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# Pharmacology of Valinate and *tert*-Leucinate Synthetic Cannabinoids 5F-AMBICA, 5F-AMB, 5F-ADB, AMB-FUBINACA, MDMB-FUBINACA, MDMB-CHMICA, and Their Analogues

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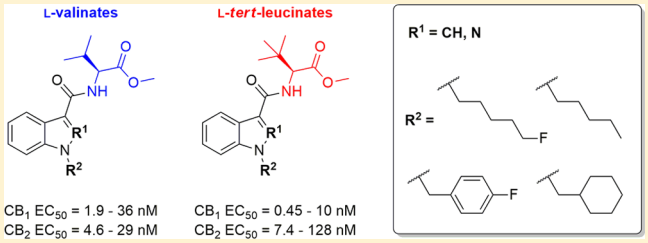
Supporting Information

**ABSTRACT:** Indole and indazole synthetic cannabinoids (SCs) featuring L-valinate or L-*tert*-leucinate pendant group have recently emerged as prevalent recreational drugs, and their use has been associated with serious adverse health effects. Due to the limited pharmacological data available for these compounds, 5F-AMBICA, 5F-AMB, 5F-ADB, AMB-FUBINACA, MDMB-FUBINACA, MDMB-CHMICA, and their analogues were synthesized and assessed for cannabimimetic activity in vitro and in vivo. All SCs acted as potent, highly efficacious agonists at $CB_1$ ($EC_{50}$ = 0.45−36 nM) and $CB_2$ ($EC_{50}$ = 4.6−128 nM) receptors in a fluorometric assay of membrane potential, with a general preference for $CB_1$ activation. The cannabimimetic properties of two prevalent compounds with confirmed toxicity in humans, 5F-AMB and MDMB-FUBINACA, were demonstrated in vivo using biotelemetry in rats. Bradycardia and hypothermia were induced by 5F-AMB and MDMB-FUBINACA doses of 0.1−1 mg/kg (and 3 mg/kg for 5F-AMB), with MDMB-FUBINACA showing the most dramatic hypothermic response recorded in our laboratory for any SC (>3 °C at 0.3 mg/kg). Reversal of hypothermia by pretreatment with a $CB_1$, but not $CB_2$, antagonist was demonstrated for 5F-AMB and MDMB-FUBINACA, consistent with $CB_1$-mediated effects in vivo. The in vitro and in vivo data indicate that these SCs act as highly efficacious CB receptor agonists with greater potency than $\Delta^9$-THC and earlier generations of SCs.




**KEYWORDS:** *Cannabinoid, THC, JWH-018, AMB, MDMB*

Synthetic cannabinoids (SCs) are the most rapidly growing class of "designer drugs", or new psychoactive substances (NPSs).[1] Consumer products available since about 2004 and intended as "legal cannabis substitutes" were found in 2008 to contain JWH-018 (**1**, Figure 1) and CP 47,497-C8 (**2**).[2,3] In 2014, 177 different SCs were reported to the United Nations Office on Drugs and Crime (UNODC) Early Warning Advisory (EWA).[4] Many novel SCs have already been discovered in 2016, and the structural diversity of these substances is increasing.[5−14]

SCs are typically found to function as agonists of cannabinoid receptor type 1 ($CB_1$) and type-2 ($CB_2$), with activation of the former accounting for the psychoactivity of these substances.[15] However, many SCs are unknown prior to first detection by forensic chemists, and nothing is known of their activity in humans. The scarcity of data regarding the pharmacological and toxicological properties of emergent SCs poses an ongoing challenge for scientists, healthcare workers, and lawmakers across the globe.[16−26]

We have previously described the in vitro and in vivo pharmacology of SCs based on 3-benzoylindoles (e.g., RCS-4, **3**), 3-naphthoylindoles (e.g., AM-2201, **4**), 3-alkanoylindoles (e.g., XLR-11, **5**), indole-3-carboxylates (e.g., 5F-PB-22, **6**), and indole-3-carboxamides (e.g., STS-135, **7**).[27−32] One of the most prevalent, recent groups of SCs are 1-alkyl-1*H*-indazole-3-carboxamides featuring pendant valinamide and *tert*-leucinamide groups, exemplified by AB-FUBINACA (**8**) and ADB-PINACA (**9**), respectively. Following the designation of several members of this class as Schedule I substances by the Drug Enforcement Administration (DEA) in the United States

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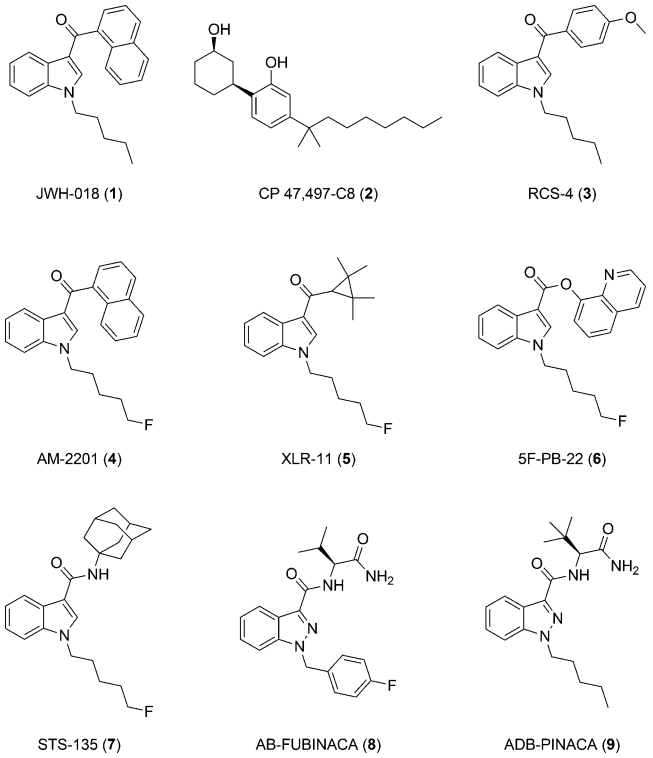


**Figure 1.** Selected synthetic cannabinoids.

(US),[33,34] newer indole- and indazole-3-carboxamide variants have appeared featuring currently popular 5-fluoropentyl, 4-fluorobenzyl, cyclohexylmethyl, or pentyl substituents at the 1-position, and valinate and *tert*-leucinate methyl ester side chains (**10**–**25**, Figure 2).

5F-AMB-PICA (MMB-2201, 5F-AMBICA, **10**) was reported to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) following its identification in Hungary and Sweden in 2014, and its indazole analogue, 5F-AMB-PINACA (5F-AMB, **12**), was found in Hungary contemporaneously.[35] The *tert*-leucinate analogue of **12**, 5F-MDMB-PINACA (5F-ADB, **13**), was notified to the UNODC EWA in Hungary and Japan in 2015. The indole derivative MDMB-FUBICA (**15**) was discovered in Hungary and Sweden in 2015, according to UNODC EWA, and AMB-FUBINACA (**16**) was reported to the EMCDDA in Sweden around the same time.[35] MDMB-FUBINACA (**17**) was identified following media monitoring by EMCDDA after it was responsible for dozens of deaths and hundreds of hospitalizations in the Russian Federation in 2015.[35,36] MDMB-CHMICA was first identified in Hungary in 2014, but has since been reported to the EWA in France, Mauritius, Serbia, Turkey, and the UK.[35]

SC use is associated with serious adverse reactions,[37−53] and the most recent SCs appear to possess greater dependence liabilities[54−57] and toxicities[58−64] than earlier examples. AMB-FUBINACA was clinically confirmed in a case of rhabdomolysis,[65] and fatal intoxications have been attributed to consumption of 5F-AMB,[44,66,67] 5F-ADB,[68] and MDMB-CHMICA.[69−71]

Aspects of the spectral properties of selected members of this class of SCs have been reported,[72−76] and details of the metabolism of AMB-PICA and 5F-AMB were recently published,[77] but little is known about the pharmacology of these compounds in vitro or in vivo.

A systematic library of indole and indazole SCs featuring a valinate or *tert*-leucinate functional group was prepared and screened for cannabinoid activity in vitro and in vivo, in order to elucidate the hitherto unknown structure−activity relationships within this class.

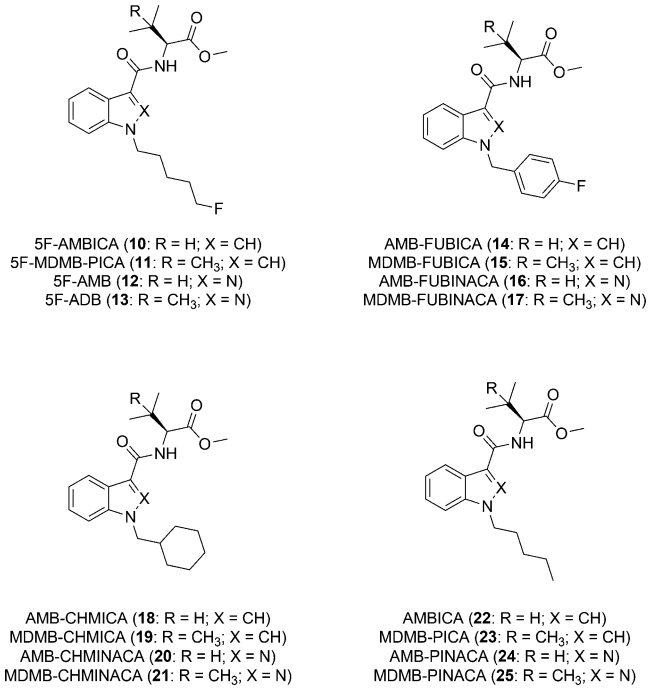


**Figure 2.** Emergent indole and indazole SCs featuring pendant methyl valinate and methyl *tert*-leucinate functional groups.

## RESULTS AND DISCUSSION

The synthesis of indole and indazole SCs required a different strategy for each heteroaromatic core. The synthesis of indole SCs **10**, **11**, **14**, **15**, **18**, **19**, **22**, and **23** is shown in Scheme 1, and the synthesis of indazole SCs **12**, **13**, **16**, **17**, **20**, **21**, **24**, and **25** is shown in Scheme 2.

As shown in Scheme 1, indole was subjected to a one-pot procedure in the presence of excess base whereby *N*-alkylation with the appropriate alkyl bromide was followed treatment with trifluoroacetic anhydride and gave trifluoroacetylindoles **27**–**30**. Base-mediated hydrolysis of the trifluoroacetyl groups of **27**–**30** gave the corresponding carboxylic acids **31**–**34**. Finally, amide bond formation was achieved by subjecting **31**–**34** to HOBt/EDC coupling with methyl L-valinate or methyl *tert*-L-leucinate to afford **10**, **11**, **14**, **15**, **18**, **19**, **22**, and **23**.

As depicted in Scheme 2, the synthesis of indazole analogues started from methyl 1*H*-indazole-3-carboxylate (**35**), which was regioselectively alkylated with the suitable bromoalkane to give the 1-alkyl-1*H*-indazole-3-carboxylate methyl esters **36**–**39**. Saponification of esters **36**–**39** afforded the corresponding acids **40**–**43**, which were coupled to methyl L-valinate or methyl *tert*-L-leucinate using the HOBt/EDC method described above, to furnish 1-alkyl-1*H*-indazole-3-carboxamides **12**, **13**, **16**, **17**, **20**, **21**, **24**, and **25**.

The activity of synthesized indole and indazole SCs **10**–**25** at $CB_1$ and $CB_2$ receptors was assessed in a fluorometric imaging plate reader (FLIPR) assay to elucidate structure−activity relationships (SARs) for this class. The activities of **10**–**25** at $CB_1$ and $CB_2$ were compared to phytocannabinoid $\Delta^9$-THC (a low efficacy agonist at $CB_1$ and $CB_2$), and CP 55,940 (an


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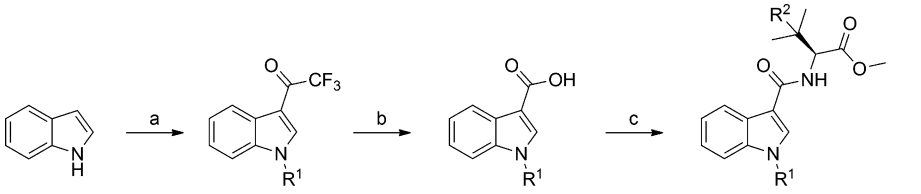
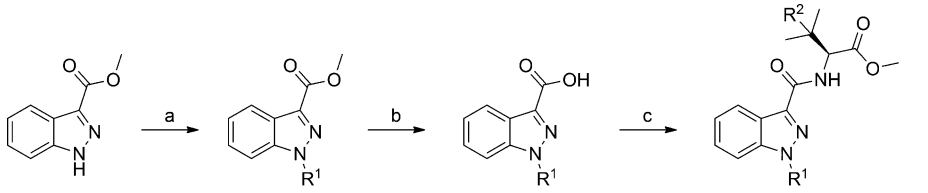
**Scheme 1. Synthesis of Indole SCs 10, 11, 14, 15, 18, 19, 22, and 23**[a]




[a]Reagents and conditions: (a) (i) NaH, BrR[1], DMF, 0 °C−rt, 1 h; (ii) $(CF_3CO)_2O$, DMF, 0 °C−rt, 1 h, 72−94%; (b) 1 M aq. NaOH, MeOH, reflux, 24 h, 67−92%; (c) methyl L-valinate or methyl L-*tert*-leucinate, EDC·HCl, HOBt, DIPEA, DMSO, rt, 24 h, 63−81%.

**Scheme 2. Synthesis of Indazole SCs 12, 13, 16, 17, 20, 21, 24, and 25**[a]




[a]Reagents and conditions: (a) *t*-BuOK, BrR[1], THF, 0 °C−rt, 48 h; 65−84%; (b) 1 M aq. NaOH, MeOH, reflux, 24 h, 76−91%; (c) methyl L-valinate or methyl L-*tert*-leucinate, EDC·HCl, HOBt, DIPEA, DMSO, rt, 24 h, 60−77%.

**Table 1. Functional Activity of $\Delta^9$-THC, CP 55,940, and Novel SCs 10−25 at $CB_1$ and $CB_2$ Receptors**

| | hCB$_1$ | | hCB$_2$ | | |
|---|---|---|---|---|---|
| compd | pEC$_{50}$ ± SEM (EC$_{50}$, nM) | max ± SEM (% CP 55,940) | pEC$_{50}$ ± SEM (EC$_{50}$, nM) | max ± SEM (%CP 55,940) | CB$_1$ sel.[a] |
| $\Delta^9$-THC | 6.77 ± 0.05 (171) | 50 ± 11 | | 20 ± 3 at 10 μM | |
| CP 55,490 | 7.47 ± 0.05 (42) | | 7.17 ± 0.07 (68) | | 1.6 |
| 5F-AMBICA (**10**) | 8.62 ± 0.06(2.4) | 107 ± 4 | 8.34 ± 0.07 (4.6) | 94 ± 3 | 1.9 |
| 5F-MDMB-PICA (**11**) | 9.35 ± 0.07(0.45) | 110 ± 4 | 8.13 ± 0.05 (7.4) | 94 ± 3 | 16.4 |
| 5F-AMB (**12**) | 8.71 ± 0.04(1.9) | 109 ± 3 | 7.99 ± 0.13 (10) | 103 ± 7 | 5.3 |
| 5F-ADB (**13**) | 9.23 ± 0.11(0.59) | 108 ± 5 | 8.12 ± 0.06 (7.5) | 94 ± 3 | 12.7 |
| AMB-FUBICA (**14**) | 7.45 ± 0.05(36) | 106 ± 3 | 7.85 ± 0.09 (14) | 86 ± 4 | 0.4 |
| MDMB-FUBICA (**15**) | 8.57 ± 0.05(2.7) | 109 ± 3 | 7.60 ± 0.12 (25) | 92 ± 6 | 9.3 |
| AMB-FUBINACA (**16**) | 8.71 ± 0.10(2.0) | 103 ± 5 | 7.75 ± 0.05 (18) | 92 ± 3 | 9.0 |
| MDMB-FUBINACA (**17**) | 8.41 ± 0.04 (3.9) | 108 ± 3 | 7.26 ± 0.14 (55) | 101 ± 9 | 14.1 |
| AMB-CHMICA (**18**) | 8.45 ± 0.08(3.5) | 114 ± 4 | 7.93 ± 0.07 (12) | 88 ± 4 | 3.4 |
| MDMB-CHMICA (**19**) | 8.00 ± 0.05 (10) | 112 ± 3 | 7.15 ± 0.05 (71) | 103 ± 3 | 7.1 |
| AMB-CHMINACA (**20**) | 8.29 ± 0.07(5.1) | 109 ± 4 | 7.54 ± 0.13 (29) | 92 ± 7 | 5.7 |
| MDMB-CHMINACA (**21**) | 7.99 ± 0.04 (10) | 111 ± 2 | 6.89 ± 0.04 (128) | 96 ± 3 | 12.8 |
| AMBICA (**22**) | 7.74 ± 0.10(18) | 111 ± 6 | 7.63 ± 0.08 (23) | 90 ± 4 | 1.3 |
| MDMB-PICA (**23**) | 8.77 ± 0.06 (1.7) | 109 ± 4 | 7.78 ± 0.13 (17) | 90 ± 5 | 10 |
| AMB-PINACA (**24**) | 8.48 ± 0.05 (3.3) | 110 ± 3 | 7.79 ± 0.11 (16) | 96 ± 5 | 4.8 |
| MDMB-PINACA (**25**) | 8.84 ± 0.06 (1.4) | 112 ± 4 | 7.56 ± 0.06 (28) | 91 ± 4 | 20 |

[a]$CB_1$ selectivity expressed as the ratio of $CB_1$ $EC_{50}$ to $CB_2$ $EC_{50}$.



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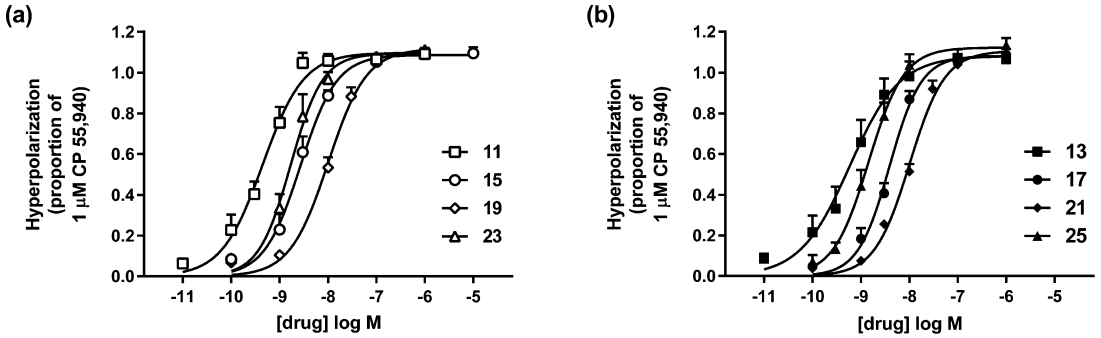


**Figure 3.** Hyperpolarization mediated by $CB_1$ receptors induced by differently 1-substituted (a) indoles **11**, **15**, **19**, and **23**, and the corresponding (b) indazoles **13**, **17**, **21**, and **25** as a proportion of that produced by 1 $\mu$M CP 55,940. Membrane potential was measured using a fluorescent dye, as outlined in the Methods. Each point represents the mean ± SEM of at least five independent determinations, each performed in duplicate. Data was fitted with a four-parameter logistic equation in GraphPad Prism.

efficacious agonist at $CB_1$ and $CB_2$), in an assay of CB receptor-dependent membrane hyperpolarization (Table 1). The assay used murine AtT20-FlpIn neuroblastoma cells stably expressing human $CB_1$ or $CB_2$ receptors. Activation of CB receptors resulted in opening of endogenous G protein-gated inwardly rectifying potassium channels (GIRKs) that produced a hyperpolarization of the cells which was reflected in a decrease in the fluorescence of a proprietary membrane potential dye. The maximum effects of $\Delta^9$-THC and **10**–**25** were compared to the high efficacy $CB_1/CB_2$ agonist CP 55,490. Data for each experiment were normalized to the change in fluorescence produced by a maximally effective concentration of CP 55,940 (1 $\mu$M). CP 55,940 (1 $\mu$M) decreased fluorescence by 31 ± 1% in CB1-expressing cells, and 26 ± 1% in CB2 expressing cells (n = 18 each). None of **10**–**25** produced a significant change in the membrane potential of wild type AtT-20 cells (n = 5 each, data not shown), which do not express $CB_1$ or $CB_2$ receptors. Consistent with a predominant coupling of $CB_1$ and $CB_2$ receptors to $G_i/G_o$ family G proteins, the effects of **10**–**25** were abolished by overnight treatment of the cells with pertussis toxin (200 ng/mL), which blocks the coupling of GPCR to $G_i/G_o$ family G proteins (n = 3 each, data not shown).

All indole and indazole SCs **10**–**25** activated $CB_1$ and $CB_2$ receptors. All compounds had greater potency (0.45–36 nM) than either $\Delta^9$-THC (171 nM) or CP 55,940 (42 nM) for $CB_1$ receptor-mediated activation of GIRK. Consistent with our previous studies using this assay, $\Delta^9$-THC was found to be a low efficacy agonist at $CB_2$ receptors, and its effects on GIRK activation in AtT20-$CB_2$ at 10 $\mu$M were only 20 ± 3% of that mediated by a maximally effective concentration of CP 55,940 (1 $\mu$M). Compounds **10**–**25** had a similar maximal effect to CP 55,940 at $CB_1$ and $CB_2$ receptors, suggesting that these SCs are also high efficacy agonists. Excluding **14**, all SCs showed a preference for $CB_1$ receptors over $CB_2$ receptors, ranging from low (e.g., **22**; 1.3 times) to moderate (e.g., **25**; 20 times). The psychoactivity of cannabinoids is attributed to activation of $CB_1$ receptors,[15] and our data are consistent the anecdotally reported psychoactive effects of members of this class of SCs.

With the exception of several pairs of nearly equal potency (**16** and **17**; **20** and **21**), all *tert*-leucinate-functionalized SCs were more potent $CB_1$ agonists than the corresponding valinate analogues, a trend that was also observed for *tert*-leucinimde and valinamide analogues in our previous work.[30]

$CB_1$ $EC_{50}$ values for **10**–**25** ranged from 0.45 to 36 nM, but only two of the 16 SCs had $EC_{50}$ values greater than 10 nM (**14** and **22**), and two demonstrated subnanomolar potencies (**11** and **13**). The least potent SC in this class (AMB-FUBICA; **14**) was roughly 4 times more potent than $\Delta^9$-THC at $CB_1$ receptors, while the most potent compound (5F-MDMB-PICA; **11**) was 380 times more potent than $\Delta^9$-THC.

Consistent with our previous work on other indole and indazole SCs, there were no obvious trends for differences in potency or efficacy when moving between these heteroaromatic cores for corresponding pairs of compounds. However, within the *tert*-leucinate-flunctionalized compounds, the nature of *N*-alkyl substituent had a consistent effect on $CB_1$ potency for compounds containing either an indole or indazole core.

For the *tert*-leucinate functionalized indoles, $CB_1$ potency decreased as a function of *N*-alkyl substituent in the order of 5-fluoropentyl (**11**), pentyl (**23**), 4-fluorobenzyl (**15**), and cyclohexylmethyl (**19**), and this trend is depicted in Figure 3a. The same trend was found for corresponding indazoles **13**, **25**, **17**, and **21**, respectively (Figure 3b). Although no such clear trend was evident for valinate-containing SCs, it is notable that three of the five least potent SCs contained a cyclohexylmethyl group at the 1-position (**19**, **20**, and **21**), and three of the five most potent SCs contained a 5-fluoropentyl substituent (**11**, **12**, and **13**) regardless of heteroaromatic core or amino acid ester side-chain.

Having demonstrated that **10**–**25** are potent and efficacious cannabimimetic agents in vitro, we sought to demonstrate activity of several of the most prevalent and toxic SCs in vivo. Both 5F-AMB and MDMB-FUBINACA have been linked with numerous incidents of adverse effects, including death, in humans.[35,36,44,66,67] The in vivo activity of 5F-AMB (**12**) and MDMB-FUBINACA (**17**) were compared using biotelemetry in rats to provide information regarding the activity of these newer SCs in a living system. Biotelemetry provides a high resolution, high fidelity alternative to the classical cannabinoid tetrad, and has the capacity to show both the magnitude and time-course of cannabinoid effects on rodent physiology.

In rodents, cross-substitution of older SCs, like JWH-018, and $\Delta^9$-THC has been demonstrated, indicating that these classes produce similar pharmacological effects despite structural dissimilarity.[78−81] Cannabinoids induce hypothermia and bradycardia in rats, and these physiological changes are common to phytocannabinoids like $\Delta^9$-THC and structurally distinct indole and indazole SCs.[82−84] We have previously determined the hypothermic and bradycardic potencies of $\Delta^9$-THC and numerous structurally diverse SCs, including JWH-018, AM-2201, UR-144, XLR-11, APICA, STS-135, PB-22, 5F-


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PB-22, AB-PINACA, and AB-FUBINACA in rats.[27,28,30] The cannabimimetic activities of 5F-AMB and MDMB-FUBINACA were assessed using radiotelemetry in male Long Evans rats, and the effects of these SCs on body temperature (Figure 4) and heart rate (Figure 5) are presented below.

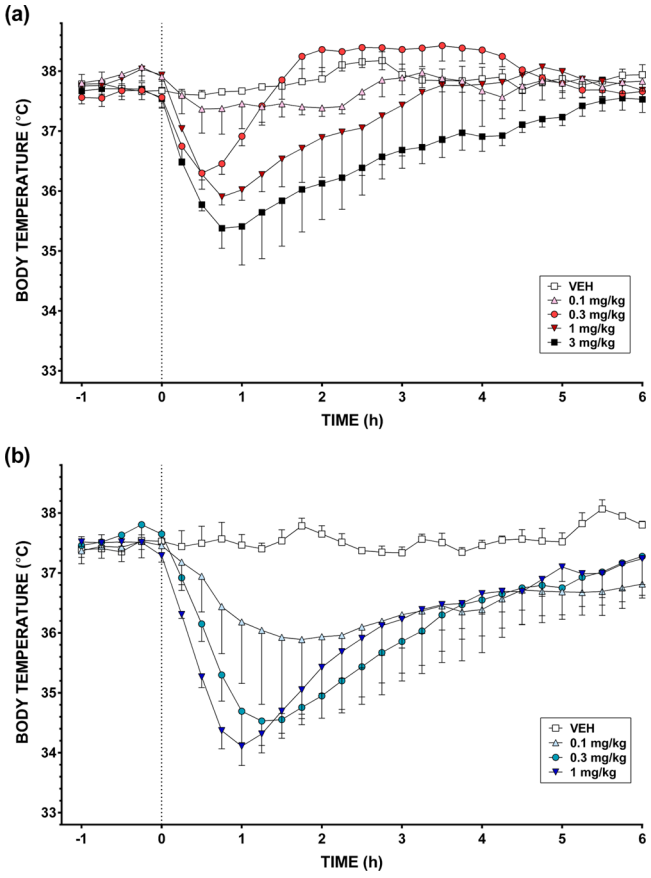


**Figure 4.** Effects of (a) 5F-AMB and (b) MDMB-FUBINACA on rat body temperature. Dashed line denotes time of intraperitoneal injection. Each point represents the mean ± SEM for four animals.

Rat body temperatures 1 h prior to intraperitoneal (i.p.) injection and 6 h post injection of 5F-AMB and MDMB-FUBINACA are presented in 15 min bins in Figure 4. For each drug, these data are presented for 1 h before (baseline) and 6 h after injection of various doses. The dashed line on the figures represents the time of SC injection. Each SC was investigated using a cohort of 3−4 rats, with a different cohort used for the two compounds. Doses were escalated from 0 mg/kg (baseline) to 0.1, 0.3, 1, and 3 mg/kg for each compound with at least 2 washout days with no injections between each dose.

Both 5F-AMB and MDMB-FUBINACA evoked a substantial hypothermic effect at doses of 0.1−1 mg/kg, and up to 3 mg/kg in the case of 5F-AMB (Figure 4). The peak reduction in body temperature was generally greater with MDMB-FUBINACA (>3 °C) than 5F-AMB (>2 °C). The hypothermic effects of MDMB-FUBINACA were so dramatic at a dose of 0.3 mg/kg, and differed so little from the increased dose of 1 mg/kg, that no higher doses were explored. These data indicate that MDMB-FUBINACA is one of the most potent SCs evaluated in rats in our laboratories thus far. Interestingly, the 0.1 mg/kg dose of MDMB-FUBINACA produced a strong hypothermic response (>3 °C) in two of the four rats tested,

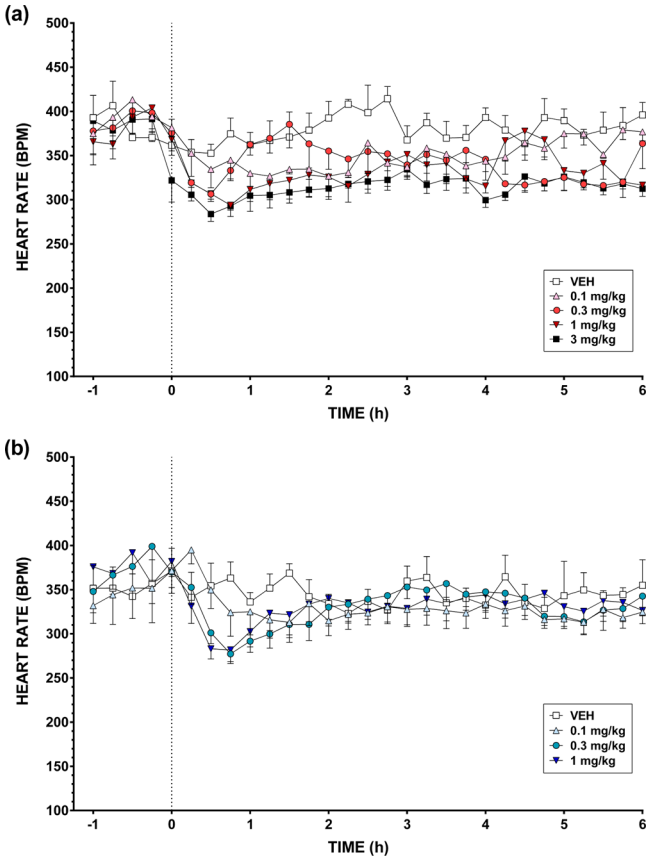


**Figure 5.** Effects of (a) 5F-AMB and (b) MDMB-FUBINACA on rat heart rate. Dashed line denotes time of intraperitoneal injection. Each point represents the mean ± SEM for four animals.

while the remaining pair did not respond. At higher doses, all four rats responded consistently. When compared to 5F-AMB, MDMB-FUBINACA induced a prolonged hypothermia at all doses, with mean core body temperature returning to baseline after more than 8 h at the smallest dose tested (0.1 mg/kg, data shown in SI). This was verified by a statistical analysis showing a significantly greater area under the curve for body temperature (relative to vehicle baseline) for MDMB-FUBINACA doses compared to 5F-AMB at 0.3 mg/kg ($P < 0.05$) and 1 mg/kg ($P < 0.05$) (see Figure S33, Supporting Information).

Data for heart rate changes effected by 5F-AMB and MDMB-FUBINACA are presented in 15 min bins in Figure 5, with the dashed line on the figures representing time of SC injection. A two-way mixed-model ANOVA with planned contrasts revealed that 5F-AMB produced a significant decrease in heart rate over the 6 h immediately following dosing at 0.3 mg/kg ($P < 0.05$), 1 mg/kg ($P < 0.05$), and 3 mg/kg ($P < 0.01$) compared to vehicle. MDMB-FUBINACA did not significantly reduce heart rate compared to vehicle at any dose over the same time period. However, heart rate was reduced in the first 2 h following injection with 0.3 mg/kg MDMB-FUBINACA ($P < 0.05$). It should be noted that heart rate data were generally more variable than those for body temperature. Variability in heart rate data is expected due to multiple determinants; locomotor activity, stress, and direct pharmacological cardiovascular effects.

To confirm that the observed effects were mediated through $CB_1$ or $CB_2$ receptors, the reversibility of the effects of 5F-AMB


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and MDMB-FUBINACA on body temperature and heart rate in rats following pretreatment with either $CB_1$ receptor antagonist rimonabant or $CB_2$ receptor antagonist SR144528 was assessed. Rimonabant is a potent and selective $CB_1$ receptor antagonist, and reverses $CB_1$-mediated cannabinoid agonist effects in rodents[85] and humans,[15] while SR144528 is a selective $CB_2$ functional antagonist.[86]

Rat body temperatures after injection (i.p.) with vehicle, $CB_1$ antagonist (rimonabant, 3 mg/kg), or $CB_2$ antagonist (SR144528, 3 mg/kg) 30 min prior to treatment with either 5F-AMB (3 mg/kg) or MDMB-FUBINACA (1 mg/kg) are presented in 15 min bins in Figure 6. For each treatment

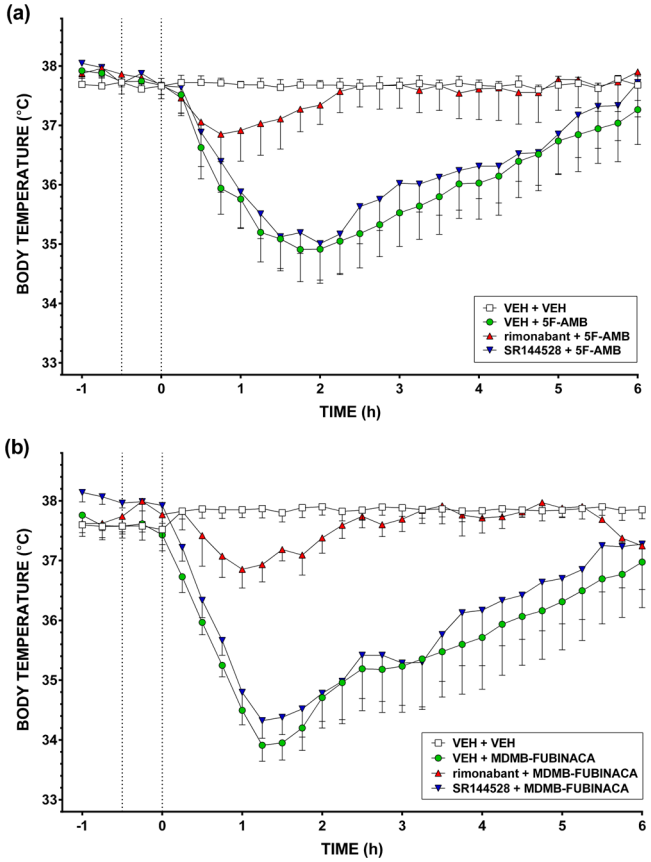


**Figure 6.** Effects of (a) 3 mg/kg 5F-AMB or (b) 1 mg/kg MDMB-FUBINACA on rat body temperature following pretreatment (30 min prior) with vehicle (VEH), 3 mg/kg rimonabant ($CB_1$ antagonist), or 3 mg/kg SR144528 ($CB_2$ antagonist). The first dashed line denotes time of intraperitoneal injection of vehicle or antagonist. Second dashed line represents time of intraperitoneal injection of SC. Each point represents the mean ± SEM for three animals.

condition, the data are presented for 1 h before (baseline) and 6 h after injection of various doses. The first dashed line on the figure represents the time of vehicle/antagonist injection, and the second dashed line represents time of SC injection. Each SC was investigated using a cohort of 3−4 rats, with a different cohort used for the two compounds.

Rimonabant pretreatment completely reversed the body temperature decrease induced by 5F-AMB or MDMB-FUBINACA, while pretreatment with SR144528 had no effect on the hypothermic effects of 5F-AMB or MDMB-FUBINACA (Figure 6a). These interpretations are confirmed by a statistical analysis of the areas between each drug treatment and baseline (Figure S35, Supporting Information), and suggest a $CB_1$-mediated hypothermic mechanism. Similar trends were observed for the reversal of 5F-AMB- or MDMB-FUBINACA-induced bradycardia by rimonabant but not SR144528, however, these differences did not reach significance (data not shown). This is likely due to a combination of the relatively smaller magnitude of SC-induced bradycardic effects and high variability of the heart rate data.

## CONCLUSION

The proactive pharmacological evaluation of emergent SCs is essential to harm minimization and law enforcement efforts targeting these compounds. This study is the first to pharmacologically characterize the most recent, prevalent class of SC designer drugs based on 1-alkylindole-3-carboxamide and 1-alkyl-1*H*-indazole-3-carboxamide scaffolds featuring pendant methyl L-valinate or methyl L-*tert*-leucinate functional groups. Synthetic routes to identified SCs of forensic interest (5F-AMBICA, 5F-AMB, AMB-FUBINACA, MDMB-FUBINACA, MDMB-CHMICA), as well as several anticipated but hitherto undetected analogues, were developed. These synthetic routes are general for 1-alkyl-1*H*-indole-3-carboxamides and 1-alkyl-1*H*-indazole-3-carboxamides and facilitate the proactive development of reference standards for SCs expected to appear in future. All synthesized SCs acted as agonists of $CB_1$ and $CB_2$ receptors in the nanomolar range in a FLIPR membrane potential assay, and are potent, functional cannabinoids. In rats, 5F-AMB and MDMB-FUBINACA dose-dependently effected hypothermia and bradycardia at doses of 0.1−1 mg/kg (and up to 3 mg/kg in the case of the former), demonstrating that these SCs are potently cannabimimetic in vivo. The dramatic reduction of body temperature induced by MDMB-FUBINACA at doses as low as 0.1 mg/kg positions this compounds as one of the most potent SCs explored in our laboratories. The hypothermic effects of 5F-AMB (3 mg/kg) and MDMB-FUBNACA (1 mg/kg) could be reversed by pretreatment with $CB_1$ antagonist rimonabant (3 mg/kg), but not $CB_2$ antagonist SR144528 (3 mg/kg), and appear to be mediated through $CB_1$ receptors. Taken together, in vitro and in vivo data confirm that SCs **10**−**25** are cannabimimetic agents of greater potency than $\Delta^9$-THC and earlier SCs.

## METHODS

**General Chemical Synthesis Details.** All reactions were performed under an atmosphere of nitrogen or argon unless otherwise specified. Commercially available chemicals were used as purchased. Analytical thin-layer chromatography was performed using Merck aluminum-backed silica gel 60 F254 (0.2 mm) plates (Merck, Darmstadt, Germany), which were visualized using shortwave (254 nm) UV fluorescence. Flash chromatography was performed using Merck Kieselgel 60 (230−400 mesh) silica gel. Melting point ranges (m.p.) were measured in open capillaries using a Stuart SMP10 melting point apparatus (Bibby Scientific, Staffordshire, UK) and are uncorrected. Nuclear magnetic resonance spectra were recorded at 300 K using either a Bruker AVANCE DRX400 (400.1 MHz) or AVANCE III 500 Ascend (500.1 MHz) spectrometer (Bruker, Bremen, Germany). The data are reported as chemical shift ($\delta$ ppm) relative to the residual protonated solvent resonance, relative integral, multiplicity (s = singlet, br s = broad singlet, d = doublet, t = triplet, quart. = quartet, quin. = quintet, m = multiplet), coupling constants ($J$ Hz), and assignment. Assignment of signals was assisted by correlation spectroscopy (COSY), distortionless enhancement by polarization transfer (DEPT), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple-bond correlation (HMBC) experiments where necessary. Low-resolution mass spectra (LRMS) was recorded


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using electrospray ionization (ESI) recorded on a Finnigan LCQ ion trap mass spectrometer (ThermoFisher Scientific, Waltham, MA). High-resolution mass spectra (HRMS) were run on a Bruker 7T Apex Qe Fourier Transform Ion Cyclotron resonance mass spectrometer equipped with an Apollo II ESI/APCI/MALDI Dual source by the Mass Spectrometry Facility of the School of Chemistry at the University of Sydney. IR absorption spectra were recorded on a Bruker ALPHA FT-IR spectrometer as solid or thin film from ethanol, and the data are reported as vibrational frequencies ($cm^{-1}$). Please see the Supporting Information for $^{1}H$ and $^{13}C$ NMR spectra and Fourier transform infrared (FTIR) spectra of all final compounds.

**General Procedure A: Amidation of 1-Alkylindole-3-carboxylic Acids and 1-Alkyl-1*H*-indazole-3-carboxylic Acids.** To a solution of the appropriate 1-alkylindole-3-carboxylic acid or 1-alkyl-1*H*-indazole-3-carboxylic acid (0.39 mmol), methyl L-valinate hydrochloride (69 mg, 0.41 mmol, 1.05 equiv) or methyl L-*tert*-leucinate hydrochloride (75 mg, 0.41 mmol, 1.05 equiv), EDC·HCl (150 mg, 0.78 mmol, 2.0 equiv), and HOBt (119 mg, 0.78 mmol, 2.0 equiv) in DMSO (5 mL) was added DIPEA (340 μL, 1.95 mmol, 5.0 equiv) dropwise and the mixture was stirred for 14 h. The reaction was quenched by the addition of sat. aq. $NaHCO_3$ (75 mL) and extracted with EtOAc (3 × 75 mL). The combined organic layers were washed with brine (100 mL), dried ($MgSO_4$), and evaporated under reduced pressure. The pure amides were obtained following purification by flash chromatography.

*Methyl (S)-2-(1-(5-Fluoropentyl)-1H-indole-3-carboxamido)-3-methylbutanoate (5F-AMB-PICA, **10**).* Subjecting **31** (100 mg, 0.40 mmol) and methyl L-valinate hydrochloride (70 mg, 0.42 mmol, 1.05 equiv) to general procedure A gave, following purification by flash chromatography (hexane-EtOAc, 80:20), **10** (92 mg, 63%) as a white solid. mp 146−148 °C; $R_f$ 0.30 (hexane/EtOAc, 80:20); $^{1}H$ NMR (300 MHz, $CDCl_3$): δ 7.96 (1H, d, *J* = 8.1 Hz), 7.70 (1H, s), 7.34 (1H, d, *J* = 8.7 Hz), 7.25−7.22 (2H, m), 6.44 (1H, d, *J* = 8.7 Hz), 4.83 (1H, dd, *J* = 8.7, 4.8 Hz), 4.46 (1H, t, *J* = 5.7 Hz), 4.30 (1H, t, *J* = 6.0 Hz), 4.12 (2H, t, *J* = 6.9 Hz), 3.75 (3H, s), 2.27 (1H, m), 1.88 (2H, quin., *J* = 7.5 Hz), 1.80−1.62 (2H, m), 1.42 (2H, quin., *J* = 8.4 Hz), 1.00 (6H, t, *J* = 6.9 Hz); $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 173.3 (CO), 165.0 (CO), 136.7 (quat.), 131.8 (CH), 125.5 (quat.), 122.7 (CH), 121.8 (CH), 120.3 (CH), 110.8 (quat.), 110.4 (CH), 83.8 ($CH_2F$, d, $^{1}J_{CF}$ = 163.5 Hz), 57.1 (CH), 52.3 ($CH_2$), 46.9 ($CH_3$), 31.9 (CH), 30.1 ($CH_2$, d, $^{2}J_{CF}$ = 20.3 Hz), 29.8 ($CH_2$), 23.0 ($CH_2$, d, $^{3}J_{CF}$ = 5.3 Hz), 19.2 ($CH_3$), 18.2 ($CH_3$); $^{19}F$ NMR (282 MHz, $CDCl_3$): − 218.6 (1F, m); LRMS (+ESI): *m*/*z* 385.14 ([M + Na]$^+$, 100%); HRMS (+ESI): *m*/*z* calculated [M + Na]$^+$ 399.2060, found 399.2054; IR (diamond cell, thin film) 3343 (w), 2977 (m), 2962 (m), 2877 (w), 1736 (s), 1625 (s), 1509 (s), 1465 (s), 1225 (s), 1198 (s), 1167 (s), 1147 (s), 751 (s).

*Methyl (S)-2-(1-(5-Fluoropentyl)-1H-indole-3-carboxamido)-3,3-dimethylbutanoate (5F-MDMB-PICA, **11**).* Subjecting **31** (100 mg, 0.40 mmol) and methyl L-*tert*-leucinate hydrochloride (76 mg, 0.42 mmol, 1.05 equiv) to general procedure A gave, following purification by flash chromatography (hexane-EtOAc, 80:20), **10** (112 mg, 74%) as a white solid. mp 82−84 °C; $R_f$ 0.35 (hexane/EtOAc, 80:20); $^{1}H$ NMR (300 MHz, $CDCl_3$): δ 7.99 (1H, m), 7.74 (1H, s), 7.38 (1H, m), 7.31−7.27 (2H, m), 7.52 (1H, d, *J* = 9.3 Hz), 4.78 (1H, d, *J* = 9.3 Hz), 4.40 (2H, dt, *J* = 41.4 Hz, 6.0 Hz), 4.15 (2H, t, *J* = 7.2 Hz), 3.76 (3H, s), 1.91 (2H, quin., *J* = 7.2 Hz), 1.81−1.62 (2H, m), 1.46 (2H, quin., *J* = 6.3 Hz), 0.93 (9H, s); $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 172.8 (CO), 164.8 (CO), 136.7 (quat.), 131.9 (CH), 125.4 (quat.), 122.6 (CH), 121.8 (CH), 120.1 (CH), 110.8 (quat.), 110.4 (CH), 83.7 ($CH_2F$, d, $^{1}J_{CF}$ = 164.3 Hz), 59.9 (CH), 51.9 ($CH_2$), 46.8 ($CH_3$), 35.2 (quat.), 30.1 ($CH_2$, d, $^{2}J_{CF}$ = 19.5 Hz), 29.7 ($CH_2$), 26.9 ($CH_3$), 22.9 ($CH_2$, d, $^{3}J_{CF}$ = 5.3 Hz); $^{19}F$ NMR (282 MHz, $CDCl_3$): δ − 218.5 (1F, m); LRMS (+ESI): *m*/*z* 399.14 ([M + Na]$^+$, 100%); HRMS (+ESI): *m*/*z* calculated [M + Na]$^+$ 399.2060, found 399.2056; IR (diamond cell, thin film) 3437 (w), 2971 (m), 2959 (m), 1730 (s), 1639 (s), 1499 (s), 1234 (s), 1186 (s), 773 (m), 751 (s).

*Methyl (S)-2-(1-(5-Fluoropentyl)-1H-indazole-3-carboxamido)-3-methylbutanoate (5F-AMB-PINACA, **12**).* Subjecting **40** (100 mg, 0.42 mmol) and methyl L-valinate hydrochloride (75 mg, 0.45 mmol, 1.1 equiv) to general procedure A gave, following purification by flash chromatography (hexane/EtOAc, 80:20), **12** (105 mg, 69%) as a white solid. mp 68−70 °C; $R_f$ 0.50 (hexane/EtOAc, 60:40); $^{1}H$ NMR (300 MHz, $CDCl_3$): δ 8.35 (1H, d, *J* = 8.1 Hz), 7.47 (1H, d, *J* = 9.0 Hz), 7.42−7.39 (2H, m), 4.80 (1H, dd, *J* = 8.7, 5.1 Hz), 4.51 (1H, t, *J* = 5.7 Hz), 4.42 (2H, t, *J* = 6.9 Hz), 4.36 (1H, t, *J* = 5.7 Hz), 3.78 (3H, s), 2.30 (1H, m, *J* = 6.6 Hz), 2.05 (2H, quin., *J* = 7.5 Hz), 1.83−1.62 (2H, m), 1.48 (2H, quin., *J* = 7.5 Hz), 1.04 (6H, t, *J* = 6.0 Hz); $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 172.8 (CO), 162.6 (CO), 141.0 (quat.), 136.9 (quat.), 126.9 (CH), 123.1 (quat.), 123.0 (CH), 122.8 (CH), 109.3 (CH), 84.0 ($CH_2$, d, $^{1}J_{CF}$ = 149.3 Hz), 56.9 (CH), 52.3 ($CH_2$), 49.3 ($CH_3$), 31.7 (CH), 30.0 ($CH_2$, d, $^{2}J_{CF}$ = 19.9 Hz), 29.5 ($CH_2$), 22.8 ($CH_2$, d, $^{3}J_{CF}$ = 5.0 Hz), 19.3 ($CH_3$), 18.2 ($CH_3$); $^{19}F$ NMR (282 MHz, CDCl3): δ − 218.5 (1F, m); LRMS (+ESI): *m*/*z* 386.11 ([M + Na]$^+$, 100%); HRMS (+ESI): *m*/*z* calculated [M + Na]$^+$ 386.1856, found 386.1849; IR (diamond cell, thin film): 3415 (w), 2960 (m), 1740 (s), 1667 (s), 1526 (s), 1491 (s), 1710 (m), 752 (m).

*Methyl (S)-2-(1-(5-Fluoropentyl)-1H-indazole-3-carboxamido)-3,3-dimethylbutanoate (5F-MDMB-PINACA, **13**).* Subjecting **40** (150 mg, 0.63 mmol) and methyl L-*tert*-leucinate hydrochloride (97 mg, 0.67 mmol, 1.05 equiv) to general procedure A gave, following purification by flash chromatography (hexane-EtOAc, 80:20), **13** (151 mg, 63%) as a white solid. mp 64−66 °C; $R_f$ 0.60 (hexane/EtOAc, 60:40); $^{1}H$ NMR (300 MHz, $CDCl_3$): δ 8.35 (1H, d, *J* = 8.1 Hz), 7.54 (1H, d, *J* = 9.6 Hz), 7.42−7.39 (2H, m), 4.73 (1H, d, *J* = 9.6 Hz), 4.52 (1H, t, *J* = 5.7 Hz), 4.42 (2H, t, *J* = 7.2 Hz), 4.36 (1H, t, *J* = 6.0 Hz), 3.76 (3H, s), 1.99 (2H, quin., *J* = 7.8 Hz), 1.84−1.67 (2H, m), 1.48 (2H, quin., *J* = 8.1 Hz), 1.09 (9H, s); $^{13}C$ NMR (300 MHz, $CDCl_3$): δ 172.3 (CO), 162.5 (CO), 141.0 (quat.), 137.0 (quat.), 126.9 (CH), 123.1 (quat.), 123.0 (CH), 122.8 (CH), 109.3 (CH), 83.9 ($CH_2$, d, $^{1}J_{CF}$ = 163.5 Hz,), 59.6 (quat.), 51.9 ($CH_2$), 49.3 ($CH_3$), 35.2 (quat.), 30.3 ($CH_2$, d, $^{2}J_{CF}$ = 19.5 Hz), 29.5 ($CH_2$), 26.8 ($CH_3$), 22.8 ($CH_2$, d, $^{3}J_{CF}$ = 4.5 Hz); $^{19}F$ NMR (282 MHz, $CDCl_3$): δ − 218.5 (1F, m); LRMS (+ESI): *m*/*z* 400.14 ([M + Na]$^+$, 100%); HRMS (+ESI): *m*/*z* calculated [M + Na]$^+$ 400.2012, found 400.2007; IR (diamond cell, thin film): 3420 (w), 2960 (m), 2870 (w), 1737 (s), 1671 (s), 1524 (s), 1491 (s), 1262 (m), 1216 (s), 752 (m).

*Methyl (S)-2-(1-(4-Fluorobenzyl)-1H-indole-3-carboxamido)-3-methylbutanoate (AMB-FUBICA, **14**).* Subjecting **32** (100 mg, 0.37 mmol) and methyl L-valinate hydrochloride (65 mg, 0.39 mmol, 1.05 equiv) to general procedure A gave, following purification by flash chromatography (hexane/EtOAc, 80:20), **14** (110 mg, 78%) as a white solid. mp 151−153 °C; $R_f$ 0.30 (hexane/EtOAc, 80:20); $^{1}H$ NMR (300 MHz, $CDCl_3$): δ 8.05 (1H, d, *J* = 7.8 Hz), 7.78 (1H, s), 7.35−7.28 (3H, m), 7.15 (2H, t, *J* = 5.4 Hz), 7.03 (2H, t, *J* = 7.5 Hz), 5.33 (2H, s), 4.90 (1H, dd, *J* = 8.7, 4.8 Hz), 3.82 (3H, s), 2.33 (1H, m, *J* = 6.0 Hz), 1.07 (6H, t, *J* = 7.2 Hz); $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 173.3 (CO), 164.9 (CO), 162.6 (quat., d, $^{1}J_{CF}$ = 247.2 Hz), 136.9 (quat.), 132.0 (CH), 131.9 (quat., d, $^{4}J_{CF}$ = 3.1 Hz), 129.0 (CH, d, $^{3}J_{CF}$ = 8.3 Hz), 125.7 (quat.), 123.0 (CH), 122.1 (CH), 120.4 (CH), 116.1 (CH, d, $^{2}J_{CF}$ = 21.9 Hz), 111.5 (quat.), 110.7 (CH), 57.1 ($CH_2$), 52.3 ($CH_3$), 50.1 (CH), 31.8 (CH), 19.2 ($CH_3$), 18.2 ($CH_3$); $^{19}F$ NMR (282 MHz, CDCl3): δ − 113.9 (1F, m); LRMS (+ESI): *m*/*z* 405.11 ([M + Na]$^+$, 100%); HRMS (+ESI): *m*/*z* 405.1590, found 405.1583; IR (diamond cell, thin film): 3327 (m), 2944 (m), 2875 (w), 1738 (s), 1630 (s), 1510 (s), 1125 (s), 766 (s), 566 (m).

*Methyl (S)-2-(1-(4-Fluorobenzyl)-1H-indole-3-carboxamido)-3,3-dimethylbutanoate (MDMB-FUBICA, **15**).* Subjecting **32** (50 mg, 0.19 mmol) and methyl L-*tert*-leucinate hydrochloride (42 mg, 0.23 mmol, 1.2 equiv) to general procedure A gave, following purification by flash chromatography (hexane/EtOAc, 80:20), **15** (58 mg, 77%) as a white solid. mp 132−134 °C; $R_f$ 0.40 (hexane/EtOAc, 80:20); $^{1}H$ NMR (300 MHz, $CDCl_3$): δ 8.02 (1H, d, *J* = 7.8 Hz), 7.77 (1H, s), 7.32−7.26 (3H, m), 7.14 (2H, t, *J* = 6.3 Hz), 7.01 (2H, t, *J* = 6.3 Hz), 6.55 (1H, d, *J* = 9.3 Hz), 5.32 (2H, s), 4.79 (1H, d, *J* = 9.6 Hz), 3.78 (3H, s), 1.10 (9H, s); $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 172.8 (CO), 164.7 (CO), 162.6 (quat., d, $^{1}J_{CF}$ = 246.0 Hz), 136.9 (quat.), 132.2 (CH), 131.9 (quat., d, $^{4}J_{CF}$ = 3.0 Hz), 128.9 (CH, d, $^{3}J_{CF}$ = 8.3 Hz), 125.6 (quat.), 123.0 (CH), 122.1 (CH), 120.2 (CH), 116.1 (CH, d, $^{2}J_{CF}$ = 21.8 Hz), 111.5 (quat.), 110.8 (CH), 59.9 ($CH_2$), 52.0 ($CH_3$), 50.1


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(CH), 35.2 (quat.), 26.9 ($CH_3$); $^{19}F$ NMR (282 MHz, $CDCl_3$): $\delta$ − 113.9 (1F, m); LRMS (+ESI): $m/z$ 419.13 ($[M + Na]^+$, 100%); HRMS (+ESI): $m/z$ calculated $[M + Na]^+$ 419.1747, found 419.1740; IR (diamond cell, thin film): 3440 (w), 2961 (w), 1725 (s), 1637 (s), 1537 (s), 1327 (s), 815 (m).

*Methyl (S)-2-(1-(4-Fluorobenzyl)-1H-indazole-3-carboxamido)-3-methylbutanoate (AMB-FUBINACA, **16**).* Subjecting **41** (100 mg, 0.37 mmol) and methyl L-valinate hydrochloride (65 mg, 0.39 /EtOAc, 90:10), **16** (85 mg, 60%) as a colorless oil. $R_f$. 0.65 (hexane/EtOAc, 80:20); $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 8.36 (1H, d, $J$ = 7.8 Hz), 7.50 (1H, d, $J$ = 9.0 Hz), 7.42−7.27 (2H, m), 7.23−7.19 (2H, t, $J$ = 6.9 Hz), 7.01 (2H, t, $J$ = 7.8 Hz), 5.61 (2H, s), 4.83 (1H, dd, $J$ = 8.7, 5.1 Hz), 3.78 (3H, s), 2.30 (1H, m, $J$ = 6.6 Hz), 1.05 (6H, t, $J$ = 5.4 Hz); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 172.7 (CO), 162.6 (quat., $^1J_{CF}$ = 245.3 Hz), 162.5 (CO), 140.9 (quat.), 137.5 (quat.), 131.9 (quat., d, $^4J_{CF}$ = 3.1 Hz), 129.1 (CH, d, $^3J_{CF}$ = 8.3 Hz), 127.2 (CH), 123.5 (quat.), 123.1 (CH), 123.0 (CH), 116.0 (CH, d, $^2J_{CF}$ = 21.8 Hz), 109.6 (CH), 56.9 (CH), 53.1 ($CH_2$), 52.3 ($CH_3$), 31.8 (CH), 19.3 ($CH_3$), 18.2 ($CH_3$); $^{19}F$ NMR (282 MHz, CDCl3): $\delta$ − 114.0 (1F, m); LRMS (+ESI): $m/z$ 406.08 ($[M + Na]^+$, 100%); HRMS (+ESI): $m/z$ calculated $[M + Na]^+$ 406.1543, found 406.1537; IR (diamond cell, thin film): 3415 (w), 2964 (m), 1740 (s), 1667 (s), 1527 (s), 1511 (s), 1492 (s), 1261 (s), 1172 (s), 1158 (s), 750 (m).

*Methyl (S)-2-(1-(4-Fluorobenzyl)-1H-indazole-3-carboxamido)-3,3-dimethylbutanoate (MDMB-FUBINACA, **17**).* Subjecting **41** (100 mg, 0.37 mmol) and methyl L-*tert*-leucinate hydrochloride (81 mg, 0.41 mmol, 1.1 equiv) to general procedure A gave, following purification by flash chromatography (hexane/EtOAc, 80:20), **17** (96 mg, 65%) as a white crystalline solid. mp 120−122 °C; $R_f$. 0.60 (hexane/EtOAc, 60:40); $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 8.21 (1H, d, $J$ = 7.8 Hz), 7.59 (1H, d, $J$ = 8.4 Hz), 7.44 (1H, t, $J$ = 7.8 Hz), 7.33−7.27 (3H, m), 7.06 (2H, t, $J$ = 8.4 Hz), 5.72 (2H, s), 4.61 (1H, s), 3.77 (3H, s), 1.09 (9H, s); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 172.3 (CO), 162.6 (quat., d, $^1J_{CF}$ = 245.3 Hz), 162.3 (CO), 140.9 (quat.), 137.5 (quat.), 131.9 (quat., d, $^4J_{CF}$ = 3.3 Hz), 129.1 (CH, d, $^3J_{CF}$ = 8.3 Hz), 127.2 (CH), 123.5 (quat.), 123.1 (CH), 123.0 (CH), 116.0 (CH, d, $^2J_{CF}$ = 21.8 Hz), 109.6 (CH), 59.7 (CH), 53.1 ($CH_2$), 51.95 ($CH_3$), 35.2 (quat.), 26.8 ($CH_3$); $^{19}F$ NMR (282 MHz, $CDCl_3$): $\delta$ − 114.00 (1F, m); LRMS (+ESI): $m/z$ 419.12 ($[M + Na]^+$, 100%); HRMS (+ESI): $m/z$ calculated $[M + Na]^+$ 420.1699, found 420.1694; IR (diamond cell, thin film): 3419 (w), 2966 (m), 1737 (s), 1670 (s), 1526 (s), 1511 (s), 1222 (s), 1166 (s), 749 (m).

*Methyl (S)-2-(1-(Cyclohexylmethyl)-1H-indole-3-carboxamido)-3-methylbutanoate (AMB-CHMICA, **18**).* Subjecting **33** (50 mg, 0.20 mmol) and methyl L-valinate hydrochloride (35 mg, 0.21 mmol, 1.05 equiv) to general procedure A gave, following purification by flash chromatography (hexane/EtOAc, 90:10), **18** (60 mg, 81%) as a white solid. mp 137−139 °C; $R_f$. 0.55 (hexane/EtOAc, 80:20); $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 7.99 (1H, m), 7.70 (1H, s), 7.38 (1H, m), 7.31−7.27 (2H, m), 6.47 (1H, d, $J$ = 8.7 Hz), 4.87 (1H, dd, $J$ = 8.7, 4.8 Hz), 3.96 (2H, d, $J$ = 7.2 Hz), 3.77 (3H, s), 2.31 (1H, m, $J$ = 5.1 Hz), 1.88 (1H, m), 1.74−1.60 (5H, m), 1.25−1.18 (3H, m), 1.06−0.98 (8H, m); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 173.3 (CO), 165.1 (CO), 137.1 (quat.), 132.7 (CH), 125.4 (quat.), 122.5 (CH), 121.6 (CH), 120.1 (CH), 110.8 (CH), 110.5 (quat.), 57.0 (CH), 53.5 ($CH_2$), 52.3 ($CH_3$), 38.7 (CH), 31.9 (CH), 31.2 ($CH_2$), 26.3 ($CH_2$), 25.8 ($CH_2$), 19.3 ($CH_3$), 18.3 ($CH_3$); LRMS (+ESI): $m/z$ 393.16 ($[M + Na]^+$, 100%); HRMS (+ESI): $m/z$ calculated $[M + Na]^+$ 393.2154, found 393.2147; IR (diamond cell, thin film): 3327 (w), 2929 (m), 2849 (w), 1735 (s), 1618 (s), 1539 (s), 1518 (s), 1257 (s), 735 (s).

*Methyl (S)-2-(1-(Cyclohexylmethyl)-1H-indole-3-carboxamido)-3,3-dimethylbutanoate (MDMB-CHMICA, **19**).* Subjecting **33** (50 mg, 0.20 mmol) and methyl L-*tert*-leucinate hydrochloride (40 mg, 0.21 mmol, 1.05 equiv) to general procedure A gave, following purification by flash chromatography (hexane/EtOAc, 90:10), **19** (55 mg, 72%) as a white solid. mp 136−138 °C; $R_f$. 0.70 (hexane/EtOAc, 80:20); $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 8.09 (1H, d, $J$ = 5.7 Hz), 8.00 (1H, s), 7.46 (1H, d, $J$ = 8.1 Hz), 7.27−7.16 (2H, m), 4.64 (1H, s), 4.04 (2H, d, $J$ = 6.9 Hz), 3.75 (3H, s), 1.89 (1H, m), 1.71−1.57 (5H, m), 1.28−1.02 (14H, m); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 172.9 (CO), 164.9 (CO), 137.1 (quat.), 132.8 (CH), 125.3 (quat.), 122.5 (CH), 121.7 (CH), 120.0 (CH), 110.8 (CH), 110.5 (quat.), 59.8 (CH), 53.5 ($CH_2$), 52.0 ($CH_3$), 38.7 (quat.), 35.2 (CH), 31.1 ($CH_2$), 26.9 ($CH_3$), 26.3 ($CH_2$), 25.8 ($CH_2$); LRMS (+ESI): $m/z$ ($[M + Na]^+$, 100%); HRMS (+ESI): $m/z$ calculated $[M + Na]^+$ 407.2311, found 407.2304; IR (diamond cell, thin film): 3320 (w), 2928 (m), 2851 (w), 1738 (s), 1614 (s), 1536 (s), 1512 (s), 1141 (s), 739 (s).

*Methyl (S)-2-(1-(Cyclohexylmethyl)-1H-indazole-3-carboxamido)-3-methylbutanoate (AMB-CHMINACA, **20**).* Subjecting **42** (75 mg, 0.29 mmol) and methyl L-valinate hydrochloride (56 mg, 0.31 mmol, 1.05 equiv) to general procedure A gave, following purification by flash chromatography (hexane/EtOAc, 80:20), **20** (83 mg, 77%) as a colorless oil. $R_f$. 0.75 (hexane/EtOAc, 60:40); $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 8.34 (1H, d, $J$ = 8.1 Hz), 7.47 (1H, d, $J$ = 9 Hz), 7.42−7.36 (2H, m), 4.80 (1H, dd, $J$ = 9.1 Hz, 5.7 Hz), 4.22 (2H, d, $J$ = 7.2 Hz), 3.78 (3H, s), 2.31 (1H, m), 2.04 (1H, m), 1.72−1.58 (5H, m), 1.30−0.97 (11H, m); $^{13}C$ NMR (300 MHz, $CDCl_3$): $\delta$ 172.7 (CO), 162.7 (CO), 141.5 (quat.), 136.7 (quat.), 126.6 (CH), 122.83 (quat.), 122.77 (CH), 122.6 (CH), 109.6 (CH), 56.8 (CH), 55.8 ($CH_2$), 52.2 ($CH_3$), 38.8 (CH), 31.7 (CH), 31.0 ($CH_2$), 26.3 ($CH_2$), 25.7 ($CH_2$), 19.2 ($CH_3$), 18.2 ($CH_3$); LRMS (+ESI): $m/z$ 394.15 ($[M + Na]^+$, 100%); HRMS (+ESI): $m/z$ calculated $[M + Na]^+$ 394.2107, found 394.2100; IR (diamond cell, thin film): 3411 (w), 2927 (s), 2852 (m), 1741 (s), 1670 (s), 1525 (s), 1491 (s), 1176 (m), 751 (m).

*Methyl (S)-2-(1-(Cyclohexylmethyl)-1H-indazole-3-carboxamido)-3,3-dimethylbutanoate (MDMB-CHMINACA, **21**).* Subjecting **42** (100 mg, 0.39 mmol) and methyl L-*tert*-leucinate hydrochloride (69 mg, 0.41 mmol, 1.05 equiv) to general procedure A gave, following purification by flash chromatography (hexane/EtOAc, 80:20), **21** (110 mg, 73%) as a colorless oil. $R_f$. 0.85 (hexane/EtOAc, 60:40); $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 8.34 (1H, d, $J$ = 8.1 Hz), 7.55 (1H, d, $J$ = 9.6 Hz), 7.40−7.36 (2H, m), 4.73 (1H, d, $J$ = 9.9 Hz), 4.22 (2H, d, $J$ = 7.2 Hz), 3.76 (3H, s), 2.03 (1H, m), 1.75−1.58 (5H, m), 1.30−1.04 (14H, m); $^{13}C$ NMR (300 MHz, $CDCl_3$): $\delta$ 172.4 (CO), 162.6 (CO), 141.5 (quat.), 136.8 (quat.), 126.7 (CH), 122.91 (quat.), 122.87 (CH), 122.6 (CH), 109.6 (CH), 59.6 (CH), 55.8 ($CH_2$), 51.9 ($CH_3$), 38.9 (quat.), 35.2 (CH), 31.1 ($CH_2$), 26.8 ($CH_3$), 26.4 ($CH_2$), 25.8 ($CH_2$); LRMS (+ESI): $m/z$ 408.17 ($[M + Na]^+$, 100%); HRMS (+ESI): $m/z$ calculated $[M + Na]^+$ 408.2263, found 408.2257; IR (diamond cell, thin film): 3417 (w), 2927 (s), 2852 (m), 1738 (s), 1672 (s), 1524 (s), 1491 (m), 1164 (m), 1134 (m), 751 (m).

*Methyl (S)-2-(1-(Pentyl)-1H-indole-3-carboxamido)-3-methylbutanoate (AMB-PICA, **22**).* Subjecting **34** (100 mg, 0.43 mmol) and methyl L-valinate hydrochloride (76 mg, 0.45 mmol, 1.1 equiv) to general procedure A gave, following purification by flash chromatography (hexane/ethyl acetate, 85:15), **22** (98 mg, 66%) as a white solid. mp 148−150 °C; $R_f$. 0.50 (hexane/ethyl acetate, 80:20); $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 7.99 (1H, d, $J$ = 6 Hz), 7.74 (1H, s), 7.38 (1H, d, $J$ = 8.7 Hz), 7.29−7.26 (2H, m), 6.48 (1H, d, $J$ = 8.4 Hz), 4.86 (1H, dd, $J$ = 8.7, 4.8 Hz), 4.13 (2H, t, $J$ = 6.9 Hz), 3.78 (3H, s), 2.31 (1H, m), 1.86 (2H, quin., $J$ = 6.9 Hz), 1.33 (4H, m), 1.04 (6H, t, $J$ = 6.9 Hz), 0.89 (3H, t, $J$ = 6.9 Hz); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 173.3 (CO), 165.1 (CO), 136.8 (quat.), 132.0 (CH), 125.5 (quat.), 122.6 (CH), 121.7 (CH), 120.2 (CH), 110.6 (quat.), 110.5 (CH), 57.0 (CH), 52.3 ($CH_3$), 47.0 ($CH_2$), 31.9 (CH), 29.8 ($CH_2$), 29.1 ($CH_2$), 22.4 ($CH_2$), 19.2 ($CH_3$), 18.2 ($CH_3$), 14.0 ($CH_3$); LRMS (+ESI): $m/z$ 367.14 ($[M + Na]^+$, 100); HRMS (+ESI): $m/z$ calculated $[M + Na]^+$ 367.1998, found 367.1992; IR (diamond cell, thin film) 3338 (w), 2952 (m), 2928 (m), 2868 (w), 1739 (s), 1630 (s), 1508 (s), 1195 (s), 1157 (s), 751 (s).

*Methyl (S)-2-(1-(Pentyl)-1H-indole-3-carboxamido)-3,3-dimethylbutanoate (MDMB-PICA, **23**).* Subjecting **34** (75 mg, 0.32 mmol) and methyl L-*tert*-leucinate hydrochloride (61 mg, 0.39 mmol, 1.2 equiv) to general procedure A gave, following purification by flash chromatography (hexane/EtOAc, 90:10), **21** (82 mg, 71%) as a white solid. mp 70−72 °C; $R_f$. 0.60 (hexane/EtOAc 80:20); $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 8.00−7.95 (2H, m), 7.75 (1H, s), 7.42−7.37 (1H, m), 7.31−7.26 (2H, m), 6.53 (1H, d, $J$ = 9.3 Hz), 4.79 (1H, d, $J$ = 9.4 Hz), 4.13 (2H, t, $J$ = 7.2 Hz), 3.76 (3H, s), 1.86 (2H, quin., $J$ = 7.1 Hz), 1.39−1.26 (4H, m), 1.09 (9H, s), 0.89 (3H, t, $J$ = 6.8 Hz); $^{13}C$ NMR


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(75 MHz, $CDCl_3$): δ 172.9 (CO), 164.9 (CO), 136.8 (quat.), 132.1 (CH), 125.4 (quat.), 122.5 (CH), 121.7 (CH), 120.1 (CH), 110.6 (quat.), 110.5 (CH), 59.8 (CH), 51.9 ($CH_3$), 47.0 ($CH_2$), 35.2 (quat.), 29.8 ($CH_2$), 29.1 ($CH_2$), 26.9 ($CH_3$), 22.4 ($CH_2$), 14.0 ($CH_3$); LRMS (+ESI): *m/z* 381.15 ($[M + Na]^+$, 100%); HRMS (+ESI): *m/z* calculated $[M + Na]^+$ 381.2154, found 381.2149; IR (diamond cell, thin film): 3434 (w), 2952 (m), 2927 (m), 2868 (w), 1732 (s), 1641 (s), 1530 (s), 1498 (s), 1214 (s), 1185 (s), 1157 (s), 1129 (s), 751 (s).

*Methyl (S)-2-(1-(Pentyl)-1H-indazole-3-carboxamido)-3-methylbutanoate (AMB-PINACA, **24**).* Subjecting **43** (100 mg, 0.46 mmol) and methyl L-valinate hydrochloride (80 mg, 0.48 mmol, 1.05 equiv) to general procedure A gave, following purification by flash chromatography (hexane/EtOAc, 85:15), **24** (108 mg, 68%) as a colorless oil. $R_f$ 0.75 (hexane/EtOAc, 60:40); $^1H$ NMR (300 MHz, $CDCl_3$): δ 8.35 (1H, d, *J* = 8.1 Hz), 7.47 (1H, d, *J* = 8.7 Hz), 7.44−7.37 (2H, m), 4.81 (1H, dd, *J* = 9.0, 5.1 Hz), 4.39 (2H, t, *J* = 7.5 Hz), 3.78 (3H, s), 2.31 (1H, m), 1.40−1.26 (4H, m), 1.04 (6H, t, *J* = 6.0 Hz), 0.90 (3H, t, *J* = 6.0 Hz); $^{13}C$ NMR (300 MHz, $CDCl_3$): δ 172.8 (CO), 162.7 (CO), 141.0 (quat.), 136.7 (quat.), 126.7 (CH), 123.1 (quat.), 122.9 (CH), 122.7 (CH), 109.4 (CH), 56.9 (CH), 52.3 ($CH_3$), 49.6 ($CH_2$), 31.7 (CH), 29.6 ($CH_2$), 29.1 ($CH_2$), 22.4 ($CH_2$), 19.3 ($CH_3$), 18.2 ($CH_3$), 14.1 ($CH_3$); LRMS (+ESI): *m/z* 368.12 ($[M + Na]^+$, 100%); HRMS (+ESI): *m/z* calculated $[M + Na]^+$ 368.1950, found 368.1944; IR (diamond cell, thin film): 3419 (w), 2959 (m), 2932 (m), 2873 (w), 1742 (s), 1670 (s), 1526 (s), 1491 (s), 1181 (s), 752 (m).

*Methyl (S)-2-(1-(Pentyl)-1H-indazole-3-carboxamido)-3,3-dimethylbutanoate (MDMB-PINACA, **25**).* Subjecting **43** (100 mg, 0.46 mmol) and methyl L-*tert*-leucinate hydrochloride (71 87 mg, 0.48 mmol, 1.05 equiv) to general procedure A gave, following purification by flash chromatography (hexane/EtOAc, 85:15), **25** (125 mg, 76%) as a colorless oil. $R_f$ 0.85 (hexane/EtOAc, 60:40); $^1H$ NMR (300 MHz, $CDCl_3$): δ 8.34 (1H, d, *J* = 8.1 Hz), 7.55 (1H, d, *J* = 9.6 Hz), 7.41−7.36 (2H, m), 4.73 (1H, d, *J* = 9.9 Hz), 4.39 (2H, t, *J* = 7.5 Hz), 3.76 (3H, s), 1.95 (2H, quin., *J* = 6.9 Hz), 1.41−1.30 (4H, m), 1.09 (9H, s), 0.90 (3H, t, *J* = 7.2 Hz); $^{13}C$ NMR (300 MHz, $CDCl_3$): δ 172.3 (CO), 162.5 (CO), 141.0 (quat.), 136.7 (quat.), 126.7 (CH), 123.1 (quat.), 122.9 (CH), 122.7 (CH), 109.4 (CH), 59.6 (CH), 51.9 ($CH_3$), 49.6 ($CH_2$), 35.2 (quat.), 29.6 ($CH_2$), 29.1 ($CH_2$), 26.8 ($CH_3$), 22.4 ($CH_2$), 14.1 ($CH_3$); LRMS (+ESI): *m/z* 382.07 ($[M + Na]^+$, 100%); HRMS (+ESI): *m/z* calculated $[M + Na]^+$ 382.2107, found 382.2101; IR (diamond cell, thin film): 3419 (bs), 2960 (m), 2873 (w), 1738 (s), 1672 (s), 1525 (s), 1492 (m), 1163 (m), 751 (m).

**General Procedure B: Synthesis of 1-Alkyl-3-(trifluoroacetyl)indoles.** To a cooled (0 °C) suspension of sodium hydride (60% dispersion in mineral oil, 137 mg, 3.42 mmol, 2.0 equiv) in DMF (6 mL) was added indole (200 mg, 1.71 mmol) portionwise and the mixture stirred for 10 min. The mixture was treated dropwise with the appropriate bromoalkane (1.80 mmol, 1.05 equiv) and stirred at ambient temperature for 1 h. The cooled (0 °C) mixture was treated dropwise with trifluoroacetic anhydride (600 μL, 4.28 mmol, 2.5 equiv) and stirred at ambient temperature for 1 h. The reaction was poured into ice water (75 mL) and extracted with $CH_2Cl_2$ (3 × 75 mL). The combined organic extracts were washed with $H_2O$ (100 mL), brine (100 mL), dried ($MgSO_4$) and the solvent evaporated under reduced pressure. The pure 1-alkyl-3-trifluoroacetylindoles were obtained following purification by flash chromatography.

*2,2,2-Trifluoro-1-(1-(5-fluoropentyl)-1H-indol-3-yl)ethanone (**27**).* Subjecting indole (500 mg, 4.27 mmol) and 1-bromo-5-fluoropentane (560 μL, 4.48 mmol, 1.05 equiv) to general prcoedure B gave, following purification by flash chromatography (hexane/EtOAc, 90:10), **27** as a red solid (920 mg, 72%). mp 42−44 °C; $R_f$ 0.53 (hexane/EtOAc, 80:20); $^1H$ NMR (300 MHz, $CDCl_3$): δ 8.42−8.39 (1H, m), 7.93 (1H, s), 7.42−7.33 (3H, m), 4.43 (2H, dt, *J* = 47.1, 5.8 Hz), 4.21 (2H, t, *J* = 6.6 Hz), 1.97 (2H, quin., *J* = 6.0 Hz), 1.74 (2H, dquin., *J* = 26.1 Hz, 5.2 Hz), 1.50 (2H, quin., *J* = 6.6 Hz); $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 174.8 (q, $^2J_{CF}$ = 34.5 Hz, quat.), 137.4 (q, $^3J_{CF}$ = 4.9 Hz, CH), 136.7 (quat.), 127.2 (quat.), 124.7 (CH), 124.0 (CH), 122.8 (CH), 117.2 (q, $^1J_{CF}$ = 289.5 Hz, $CF_3$), 110.4 (CH), 109.6 (quat.), 83.7 (d, $^1J_{CF}$ = 164.3 Hz, $CH_2$), 47.6 ($CH_2$), 29.9 (d, $^2J_{CF}$ = 19.5 Hz, $CH_2$), 29.5 ($CH_2$), 22.9 (d, $^3J_{CF}$ = 4.5 Hz, $CH_2$); $^{19}F$ NMR (282 MHz, $CDCl_3$): δ − 72.2 (3F, s), − 218.7 (1F, m); LRMS (+ESI): *m/z* 324.00 ($[M + Na]^+$, 100%); IR (diamond cell, thin film): 3154 (w), 2956 (m), 2922 (m), 2862 (w), 1658 (s), 1526 (s), 1280 (s), 1176 (s), 1133 (s), 876 (s), 759 (s), 726 (s).

*2,2,2-Trifluoro-1-(1-(4-fluorobenzyl)-1H-indol-3-yl)ethanone (**28**).* Subjecting indole (250 mg, 2.13 mmol) and 4-fluorobenzyl bromide (290 μL, 2.35 mmol, 1.1 equiv) to general prcoedure B gave, following purification by flash chromatography (hexane/EtOAc, 90:10), **28** as a red solid (518 mg, 76%). mp 82−84 °C; $R_f$ 0.57 (hexane/EtOAc, 80:20); $^1H$ NMR (300 MHz, $CDCl_3$): δ 8.40 (1H, d, *J* = 7.7 Hz), 7.95 (1H, s), 7.38−7.29 (3H, m), 7.17−7.06 (2H, m), 7.06−7.00 (2H, m), 5.35 (2H, s); $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 175.1 (q, $^2J_{CF}$ = 35.3 Hz, CO), 162.8 (d, $^1J_{CF}$ = 246.8 Hz, quat.), 137.6 (q, $^4J_{CF}$ = 4.9 Hz, CH), 136.8 (quat.), 130.7 (d, $^4J_{CF}$ = 3.2 Hz, quat.), 128.9 (d, $^3J_{CF}$ = 8.2 Hz, CH), 127.3 (quat.), 125.0 (CH), 124.3 (CH), 122.8 (CH), 117.1 (q, $^1J_{CF}$ = 291.1 Hz, quat.), 110.8 (CH), 110.1 (quat.), 50.8 ($CH_2$); $^{19}F$ NMR (282 MHz, $CDCl_3$): δ − 72.3 (3F, s), − 113.0 (1F, m); LRMS (+ESI): *m/z* 344.01 ($[M + Na]^+$, 100%); IR (diamond cell, thin film): 3130 (w), 3080 (w), 2943 (w), 2866 (w), 1654 (s), 1525 (s), 1509 (s), 1392 (s), 1281 (m), 1157 (s), 1131 (s), 1046 (s), 876 (s), 747 (s), 728 (s).

*2,2,2-Trifluoro-1-(1-(cyclohexylmethyl)-1H-indol-3-yl)ethanone (**29**).* Subjecting indole (200 mg, 1.71 mmol) and (cyclohexyl)methyl bromide (250 μL, 1.80 mmol, 1.05 equiv) to general prcoedure B gave, following purification by flash chromatography (hexane/EtOAc, 93:7), **29** as a pale brown solid (425 mg, 80%). mp 87−89 °C; $R_f$ 0.79 (hexane/EtOAc, 80:20); $^1H$ NMR (300 MHz, $CDCl_3$): δ 8.42 (1H, d, *J* = 4.1 Hz), 7.89 (1H, s), 7.45−7.32 (3H, m), 4.02 (2H, d, *J* = 6.9 Hz), 1.90 (1H, m), 1.74−1.62 (5H, m), 1.24−1.00 (5H, m); $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 174.8 (q, $^2J_{CF}$ = 34.5 Hz, CO), 138.1 (CH), 137.1 (quat.), 127.1 (quat.), 124.5 (CH), 123.9 (CH), 122.7 (CH), 117.2 (quat.), 110.7 (CH), 109.3 (quat.), 54.2 ($CH_2$), 38.3 (CH), 30.9 ($CH_2$), 26.1 ($CH_2$), 25.6 ($CH_2$); $^{19}F$ NMR (282 MHz, $CDCl_3$): δ − 72.2 (3F, s); LRMS (+ESI): *m/z* 332.06 ($[M + Na]^+$, 100%); IR (diamond cell, thin film): 3118 (w), 2925 (m), 2854 (w), 1650 (s), 1531 (s), 1397 (m), 1284 (m), 1179 (s), 1130 (s), 1048 (m), 875 (s), 748 (s), 725 (s).

*2,2,2-Trifluoro-1-(1-(pentyl)-1H-indol-3-yl)ethanone (**30**).* Subjecting indole (200 mg, 1.71 mmol) and 1-bromopentane (225 μL, 1.80 mmol, 1.05 equiv) to general prcoedure B gave, following purification by flash chromatography (hexane/ethyl acetate 94:6), **30** as a yellow oil (450 mg, 94%). $R_f$ 0.81 (hexane-ethyl acetate 80:20); $^1H$ NMR (300 MHz, $CDCl_3$): δ 8.43 (1H, d, *J* = 8.7 Hz), 7.93 (1H, s), 7.42−7.36 (3H, m), 4.21 (2H, t, *J* = 6.9 Hz), 1.95 (2H, quin., *J* = 7.2 Hz), 1.43−1.32 (4H, m), 0.92 (3H, t, *J* = 6.6 Hz); $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 174.8 (q, CO, $^2J_{CF}$ = 34.5 Hz), 137.5 (d, CH, $^4J_{CF}$ = 4.5 Hz), 136.8 (quat.), 127.3 (quat.), 124.6 (CH), 124.0 (CH), 122.8 (CH), 117.3 (q, $CF_3$, $^1J_{CF}$ = 289.5 Hz), 110.5 (CH), 109.5 (quat.), 47.8 ($CH_2$), 29.5 ($CH_2$), 29.0 ($CH_2$), 22.3 ($CH_2$), 14.0 ($CH_3$); $^{19}F$ NMR (282 MHz, $CDCl_3$): δ − 72.2 (3F, s); LRMS (+ESI): *m/z* 306.03 ($[M + Na]^+$, 100%); IR (diamond cell, thin film): 3124 (w), 2959 (m), 2933 (m), 2863 (w), 1662 (s), 1527 (s), 1397 (m), 1286 (m), 1181 (s), 1132 (s), 878 (m), 751 (m).

**General Procedure C: Synthesis of 1-Alkyl-1*H*-indole-3-carboxylic Acids and 1-Alkyl-1*H*-indazole-3-carboxylic Acids.** To a solution of the appropriate 1-alkyl-3-(trifluoroacetyl)indole or methyl 1-alkyl-1*H*-indazole-3-carboxylate (2.58 mmol) in MeOH (20 mL) was added 1 M aq. NaOH (3.87 mL, 3.87 mmol, 1.5 equiv) and the solution heated at reflux for 18 h. The mixture was cooled to ambient temperature, solvent was evaporated in vacuo, and the mixture was poured onto sat. aq. $NaHCO_3$ (75 mL). The aqueous phase was washed with $Et_2O$ (75 mL) and the pH adjusted to 2 with 1 M aq. HCl. The aquoeus phase was extracted with $Et_2O$ (3 × 75 mL) and the combined organic layers were washed with brine (150 mL), dried ($MgSO_4$) and concentrated in vacuo to give the crude products. Analytical purity for 1-alkyl-1*H*-indazole-3-carboxylic acids was achieved by recrystallization from *i*-PrOH.



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*1-(5-Fluoropentyl)-1H-indole-3-carboxylic Acid (**31**).* Subjecting **27** (500 mg, 1.66 mmol) to general procedure C gave **31** (338 mg, 82%) as a white solid. mp 120−122 °C; $^1$H NMR (75 MHz, $CD_3OD$): $\delta$ 8.08 (1H, d, $J$ = 7.8 Hz), 7.96 (1H, s), 7.48 (1H, d, $J$ = 7.8 Hz), 7.28−7.17 (2H, m), 4.38 (2H, dt, $J$ = 47.4, 6.0 Hz), 4.25 (2H, t, $J$ = 6.9 Hz), 1.91 (2H, quin., $J$ = 7.8 Hz), 1.77−1.63 (2H, dquin., $J$ = 25.8, 7.2 Hz), 1.42 (2H, quin., $J$ = 7.8 Hz); $^{13}$C NMR (75 MHz, $CD_3OD$): $\delta$ 168.8 (CO), 138.1 (quat.), 136.3 (CH), 128.3 (quat.), 123.6 (CH), 122.6 (CH), 122.5 (CH), 111.4 (CH), 107.8 (quat.), 84.6 (d, $^2J_{CF}$ = 162.8 Hz, $CH_2$), 47.6 ($CH_2$), 31.1 (d, $^3J_{CF}$ = 9.5 Hz, $CH_2$), 30.7 ($CH_2$), 23.7 ($CH_2$); $^{19}$F NMR (282 MHz, $CD_3OD$): $\delta$ − 221.8 (1F, m); LRMS (−ESI): $m/z$ 248.29 ([M − H]$^-$, 100%); IR (diamond cell, thin film): 3043 (w), 2962 (m), 2895 (w), 2704 (w), 2585 (w), 1635 (s), 1523 (s), 1467 (s), 1397 (s), 1272 (s), 1170 (s), 920 (s), 742 (s), 618 (s), 427 (s).

*1-(4-Fluorobenzyl)-1H-indole-3-carboxylic Acid (**32**).* Subjecting **28** (500 mg, 1.56 mmol) to general procedure C gave **32** (282 mg, 67%) as a white solid. mp 207−209 °C; $^1$H NMR (300 MHz, $CD_3OD$): $\delta$ 8.10 (1H, dd, $J$ = 6.3, 3.3 Hz), 8.03 (1H, s), 7.39 (1H, dd, $J$ = 5.7, 2.1 Hz), 7.26−7.19 (4H, m), 7.05 (2H, t, $J$ = 8.4 Hz), 5.43 (2H, s); $^{13}$C NMR (75 MHz, $CD_3OD$): $\delta$ 168.7 (CO), 163.8 (d, $^1J_{CF}$ = 243.8 Hz, quat.), 138.2 (quat.), 136.5 (CH), 134.2 (quat.), 130.2 (d, $^3J_{CF}$ = 8.3 Hz, CH), 128.5 (quat.), 123.8 (CH), 122.8 (CH), 122.5 (CH), 116.6 (d, $^2J_{CF}$ = 21.8 Hz, CH), 111.7 (CH), 108.5 (quat.), 50.6 ($CH_2$); $^{19}$F NMR (282 MHz, $CD_3OD$): $\delta$ − 118.3 (1F, m); LRMS (−ESI): 268.22 ([M − H]$^-$, 100%); IR (diamond cell, thin film): 3108 (w), 2938 (w), 2587 (bs), 1652 (s), 1525 (m), 1508 (m), 1277 (m), 1225 (s), 1185 (s), 830 (s), 753 (s), 744 (s), 428 (s).

*1-(Cyclohexylmethyl)-1H-indole-3-carboxylic Acid (**33**).* Subjecting **29** (400 mg, 1.29 mmol) to general procedure C gave **33** (305 mg, 92%) as a white solid. mp 180−182 °C; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.19−8.16 (1H, m), 7.81 (1H, s), 7.32−7.28 (1H, m), 7.25−7.20 (2H, m), 3.92 (2H, d, $J$ = 7.2 Hz), 1.87−1.76 (1H, m), 1.68−1.56 (5H, m), 1.19−1.03 (3H, m), 1.00−0.90 (2H, m); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 170.6 (CO), 137.1 (quat.), 136.3 (CH), 127.1 (quat.), 122.9 (CH), 122.2 (CH), 122.0 (CH), 110.5 (CH), 106.2 (quat.), 53.8 ($CH_2$), 38.5 (CH), 31.1 ($CH_2$), 26.3 ($CH_2$), 25.8 ($CH_2$); LRMS (−ESI): $m/z$ 256.52 ([M − H]$^-$, 100%); IR (diamond cell, thin film): 2926 (w), 2852 (w), 1652 (s), 1523 (m), 1467 (m), 1395 (m), 1236 (s), 754 (s), 744 (s).

*1-(Pentyl)-1H-indole-3-carboxylic Acid (**34**).* Subjecting **30** (450 mg, 1.59 mmol) to general procedure C gave **33** (323 mg, 88%) as a white solid. mp 106−108 °C; $^1$H NMR (300 MHz, $CD_3OD$): $\delta$ 8.09 (1H, dd, $J$ = 6.5, 1.9 Hz), 7.93 (1H, s), 7.42−7.28 (3H, m), 4.17 (2H, t, $J$ = 7.2 Hz), 1.90 (2H, quin., $J$ = 7 Hz), 1.40−1.32 (4H, m), 0.90 (3H, t, $J$ = 6.6 Hz); $^{13}$C NMR (75 MHz, $CD_3OD$): $\delta$ 168.8 (CO), 138.1 (quat.), 136.3 (CH), 128.3 (quat.), 123.6 (CH), 122.6 (CH), 122.4 (CH), 111.3 (CH), 107.7 (quat.), 47.7 ($CH_2$), 30.7 ($CH_2$), 30.0 ($CH_2$), 23.3 ($CH_2$), 14.2 ($CH_3$); LRMS (−ESI): $m/z$ 230.32 ([M − H]$^-$, 100%); IR (diamond cell, thin film): 3106 (w), 2925 (m), 2856 (w), 2525 (bs), 1649 (s), 1526 (s), 1461 (m), 1273 (m), 1204 (s), 1117 (m), 940 (m), 731 (s).

*1-(5-Fluoropentyl)-1H-indazole-3-carboxylic Acid (**40**).* Subjecting **36** (750 mg, 2.84 mmol) to general procedure C gave **40** (580 mg, 81%) as a white solid. mp 89−91 °C; $^1$H NMR (300 MHz, $CD_3OD$): $\delta$ 8.36 (1H, d, $J$ = 7.8 Hz), 7.52 (2H, m), 7.33 (1H, t, $J$ = 7.5 Hz), 4.53−4.49 (3H, m), 4.34 (1H, t, $J$ = 5.7 Hz), 2.03 (2H, quin., $J$ = 7.2 Hz), 1.82−1.65 (2H, m), 1.50 (2H, quin., $J$ = 6.9 Hz); $^{13}$C NMR (75 MHz, $CD_3OD$): $\delta$ 165.5 (CO), 142.2 (quat.), 136.1 (quat.), 128.1 (CH), 124.7 (quat.), 124.2 (CH), 123.0 (CH), 111.2 (CH), 84.6 (d, $^1J_{CF}$ = 162.8 Hz, $CH_2$), 50.3 ($CH_2$), 31.0 (d, $^2J_{CF}$ = 20.3 Hz, $CH_2$), 30.4 ($CH_2$), 23.6 (d, $^3J_{CF}$ = 5.3 Hz, $CH_2$); $^{19}$F NMR (282 MHz, $CD_3OD$): $\delta$ − 221.8 (1F, m); LRMS (−ESI): $m/z$ 249.12 ([M − H]$^-$, 100%); IR (diamond cell, thin film): 3052 (bs), 2941 (m), 2866 (w), 1685 (s), 1480 (s), 1167 (s), 1120 (s), 751 (s).

*1-(4-Fluorobenzyl)-1H-indazole-3-carboxylic Acid (**41**).* Subjecting **37** (560 mg, 1.97 mmol) to general procedure C gave **41** (480 mg, 91%) as a white solid. mp 203−205 °C; $^1$H NMR (300 MHz, $CD_3OD$): $\delta$ 8.17 (1H, d, $J$ = 8.1 Hz), 7.62 (1H, d, $J$ = 8.7 Hz), 7.44 (1H, t, $J$ = 7.5 Hz), 7.31 (3H, m), 7.04 (2H, t, $J$ = 8.4 Hz), 5.71 (2H, s); $^{13}$C NMR (75 MHz, $CD_3OD$): $\delta$ 165.5 (CO), 163.9 (d, $^1J_{CF}$ = 244.5 Hz, quat.), 142.1 (quat.), 136.7 (quat.), 133.7 (d, $^4J_{CF}$ = 3 Hz, quat.), 130.6 (d, $^3J_{CF}$ = 8.3 Hz, CH), 128.3 (CH), 125.0 (quat.), 124.3 (CH), 123.1 (CH), 116.5 (d, $^2J_{CF}$ = 21.8 Hz, CH), 111.4 (CH), 53.6 ($CH_2$); $^{19}$F NMR (285 MHz, $CD_3OD$): $\delta$ − 118.0 (1F, m); LRMS (−ESI): $m/z$ 269.07 ([M − H]$^-$, 100%); IR (diamond cell, thin film): 3058 (bs), 2926 (w), 1696 (s), 1510 (s), 1481 (s), 1224 (s), 1170 (s), 1157 (s), 749 (s).

*1-(Cyclohexylmethyl)-1H-indazole-3-carboxylic Acid (**42**).* Subjecting **38** (475 mg, 1.74 mmol) to general procedure C gave **42** (388 mg, 86%) as a white solid. mp 124−126 °C; $^1$H NMR (300 MHz, $CD_3OD$): $\delta$ 8.16 (1H, d, $J$ = 8.1 Hz), 7.66 (1H, d, $J$ = 8.4 Hz), 7.47 (1H, t, $J$ = 7.2 Hz), 7.31 (1H, t, $J$ = 7.8 Hz), 4.34 (2H, d, $J$ = 7.2 Hz), 2.05 (1H, m), 1.72−1.66 (3H, m), 1.56 (2H, d, $J$ = 12.9 Hz), 1.26−1.02 (5H, m); $^{13}$C NMR (75 MHz, $CD_3OD$): $\delta$ 165.5 (CO), 142.6 (quat.), 135.9 (quat.), 128.0 (CH), 124.5 (quat.), 124.1 (CH), 123.0 (CH), 111.4 (CH), 56.5 ($CH_2$), 40.0 (CH), 31.7 ($CH_2$), 27.7 (CH), 26.7 ($CH_2$); LRMS (−ESI): $m/z$ 257.16 ([M − H]$^-$, 100%); IR (diamond cell, thin film): 3060 (bs), 2926 (s), 2851 (m), 1707 (s), 1479 (s), 1230 (s), 1174 (s), 752 (s).

*1-(Pentyl)-1H-indazole-3-carboxylic Acid (**43**).* Subjecting **39** (600 mg, 2.58 mmol) to general procedure C gave **43** (510 mg, 85%) as a white solid. mp 76−78 °C; $^1$H NMR (300 MHz, $CD_3OD$): $\delta$ 8.26 (1H, d, $J$ = 8.1 Hz), 7.52−7.44 (2H, m), 7.35 (1H, t, $J$ = 7.8 Hz), 4.48 (2H, t, $J$ = 7.2 Hz), 1.99 (2H, quin., $J$ = 7.2 Hz), 1.34 (4H, m), 0.89 (3H, t, $J$ = 6.0 Hz); $^{13}$C NMR (75 MHz, $CD_3OD$): $\delta$ 165.4 (CO), 142.0 (quat.), 135.9 (quat.), 127.9 (CH), 124.5 (quat.), 124.0 (CH), 122.9 (CH), 111.0 (CH), 49.7 ($CH_2$), 30.4 ($CH_2$), 29.8 ($CH_2$), 23.1 ($CH_2$), 14.1 ($CH_3$); LRMS (−ESI): $m/z$ 231.12 ([M − H]$^-$, 100%); IR (diamond cell, thin film): 3053 (bs), 2956 (m), 2931 (m), 2860 (w), 1687 (s), 1503 (s), 1218 (s), 1176 (s), 1121 (s), 752 (s).

**General Procedure D: Synthesis of Methyl 1-Alkyl-1*H*-indazole-3-carboxylates.** To a cooled (0 °C) solution of methyl 1*H*-indazole-3-carboxylate (**35**, 500 mg, 2.84 mmol) in THF (15 mL) was added potassium *tert*-butoxide (350 mg, 3.12 mmol, 1.1 equiv), and the mixture warmed to ambient temperature and stirred for 1 h. The cooled (0 °C) mixture was treated dropwise with the appropriate bromoalkane (2.98 mmol, 1.05 equiv) and stirred for 48 h. The reaction was quenched by pouring onto $H_2O$ (100 mL) and the layers separated. The aqueous phase was extracted with EtOAc (3 × 100 mL), and the combined organic layers were washed with brine (150 mL), dried ($MgSO_4$), and the solvent evaporated under reduced pressure. The crude materials were purified by flash chromatography.

*Methyl 1-(5-Fluoropentyl)-1H-indazole-3-carboxylate (**36**).* Subjecting **35** (500 mg, 2.84 mmol) and 1-bromo-5-fluoropentane (370 $\mu$L, 2.98 mmol, 1.05 equiv) to general procedure D gave, following purification by flash chromatography (hexane/EtOAc, 80:20), **36** (560 mg, 75%) as a colorless oil. $R_f$ 0.30 (hexane/EtOAc, 80:20); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 8.24 (1H, d, $J$ = 8.1 Hz), 7.49−7.45 (2H, m), 7.32 (1H, t, $J$ = 6.3 Hz), 4.52−4.47 (3H, m), 4.33 (1H, t, $J$ = 5.7 Hz), 4.04 (3H, s), 2.03 (2H, quin., $J$ = 7.2 Hz), 2.00−1.63 (2H, m), 1.46 (2H, quin., $J$ = 12.0 Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 163.2 (CO), 140.6 (quat.), 134.8 (quat.), 127.0 (CH), 123.9 (quat.), 123.2 (CH), 122.4 (CH), 109.6 (CH), 83.8 (d, $^1J_{CF}$ = 163.5 Hz, $CH_2F$), 52.1 ($CH_2$), 49.8 ($CH_3$), 30.0 (d, $^2J_{CF}$ = 19.5 Hz, $CH_2$), 29.6 ($CH_2$), 22.8 (d, $^3J_{CF}$ = 4.5 Hz, $CH_2$); $^{19}$F NMR (282 MHz, $CDCl_3$): $\delta$ − 218.6 (1F, m); LRMS (+ESI): $m/z$ 287.03 ([M + Na]$^+$, 100%); IR (diamond cell, thin film): 2950 (m), 2867 (w), 1729 (s), 1710 (s), 1478 (s), 1163 (s), 1118 (s), 752 (s).

*Methyl 1-(4-Fluorobenzyl)-1H-indazole-3-carboxylate (**37**).* Subjecting **35** (500 mg, 2.84 mmol) and 4-fluorobenzyl bromide (371 $\mu$L, 2.98 mmol, 1.05 equiv) to general procedure D gave, following purification by flash chromatography (hexane/EtOAc, 80:20), **37** (570 mg, 71%) as a white solid. mp 83−84 °C; $R_f$ 0.35 (hexane/EtOAc, 80:20); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 8.25 (1H, d, $J$ = 7.8 Hz), 7.42−7.29 (3H, m), 7.21 (2H, t, $J$ = 6.9 Hz), 6.99 (2H, t, $J$ = 8.1 Hz), 5.67 (2H, s), 4.06 (3H, s); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 163.1 (CO), 162.6 (d, $^1J_{C-F}$ = 245.3 Hz, quat.), 140.6 (quat.), 135.3 (quat.), 131.6 (d, $^4J_{CF}$ = 3.8 Hz, quat.), 129.2 (d, $^3J_{CF}$ = 8.3 Hz, CH), 127.3 (CH), 124.3 (quat.), 123.5 (CH), 122.5 (CH), 116.0 (d, $^2J_{CF}$ = 21.8


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Hz, CH), 53.5 ($CH_2$), 52.2 ($CH_3$); $^{19}F$ NMR (282 MHz, $CDCl_3$): $\delta$ −113.8 (1F, s); LRMS (+ESI): $m/z$ 307.00 ([M+ Na]$^+$, 100%). IR (diamond cell, thin film): 3071 (w), 2952 (w), 1712 (s), 1510 (s), 1479 (s), 1268 (s), 1157 (s), 749 (s).

*Methyl 1-(Cyclohexylmethyl)-1H-indazole-3-carboxylate (**38**).* Subjecting **35** (500 mg, 2.84 mmol) and (bromomethyl)cyclohexane (415 μL, 2.98 mmol, 1.05 equiv) to general procedure D gave, following purification by flash chromatography (hexane/EtOAc, 80:20), **38** (505 mg, 65%) as a colorless oil. $R_f$ 0.60 (hexane/EtOAc, 80:20); $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 8.22 (1H, dt, $J$ = 8.1, 0.9 Hz), 7.48−7.39 (2H, m), 7.33−7.28 (1H, m), 4.28 (2H, d, $J$ = 7.5 Hz), 4.03 (3H, s), 2.08 (1H, m), 1.74−1.52 (5H, m), 1.32−0.98 (5H, m); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 163.3 (CO), 141.3 (quat.), 134.6 (quat.), 126.8 (CH), 123.7 (quat.), 123.1 (CH), 122.3 (CH), 110.0 (CH), 56.1 ($CH_2$), 52.1 ($CH_3$), 38.9 ($CH_2$), 31.7 (CH), 26.3 ($CH_2$), 25.7 ($CH_2$); LRMS (+ESI) $m/z$ 295.05 ([M + Na]$^+$, 100%); IR (diamond cell, thin film): 2925 (s), 2851 (m), 1710 (s), 1477 (s), 1441 (m), 1224 (s), 1161 (s), 1121 (s), 751 (s).

*Methyl 1-(Pentyl)-1H-indazole-3-carboxylate (**39**).* Subjecting **35** (500 mg, 2.84 mmol) and 1-bromopentane (370 μL, 2.98 mmol, 1.05 equiv) to general procedure D gave, following purification by flash chromatography (hexane/EtOAc, 80:20), **39** (585 mg, 84%) as a colorless oil. $R_f$ 0.50 (hexane-EtOAc, 80:20); $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 8.24 (1H, d, $J$ = 8.0 Hz), 7.50−7.44 (2H, m), 7.35−7.27 (1H, m), 4.47 (2H, t, $J$ = 7.4 Hz), 4.04 (3H, s), 1.97 (2H, quin., $J$ = 7.0 Hz), 1.32 (4H, m), 0.87 (3H, t, $J$ = 6.6 Hz); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 163.3 (CO), 140.6 (quat.), 134.6 (quat.), 126.8 (CH), 123.9 (quat.), 123.1 (CH), 122.3 (CH), 109.7 (CH), 52.1 ($CH_3$), 50.1 ($CH_2$), 29.7 ($CH_2$), 29.0 ($CH_2$), 22.4 ($CH_2$), 14.0 ($CH_3$); LRMS (+ESI): $m/z$ 269.03 ([M + Na]$^+$, 60%), 515.16 ([2 M + Na]$^+$, 100%); IR (diamond cell, thin film): 2954 (m), 2932 (m), 2860 (w), 1709 (s), 1477 (s), 1215 (s), 1159 (s), 1117 (s), 751 (s).

**In Vitro Pharmacological Assessment of SCs.** Mouse AtT-20 pituitary tumor cells engineered to express a FLP recombination site were transfected with HA-tagged human $CB_1$ or human $CB_2$ receptors (Genscript, Piscataway, NJ) as previously described for opioid receptors in the same cells.[87] Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 100 U penicillin/streptomycin ml$^{-1}$, and 80 μg/mL hygromycin. Wild type AtT-20 FlpIn cells were grown without hygromycin. Cells were passaged at 80% confluency as required. Cells for assays were grown in 75 cm$^2$ flasks and used at 90% confluence. The day before the assay cells were detached from the flask with trypsin/EDTA (Sigma-Aldrich) and resuspended in 10 mL of Leibovitz's L-15 media supplemented with 1% FBS, 100 U penicillin/streptomycin ml$^{-1}$ and 15 mM glucose. The cells were plated in volume of 90 μL in black walled, clear bottomed 96-well microplates (Corning, Oneonta, NY). For experiments where cells were treated with pertussis toxin (PTX), the cells were plated as normal and PTX (200 ng/mL final concentration, List Biological Laboratories, Campbell, California) was added to the wells immediately afterward. Cells were incubated overnight at 37 °C in ambient $CO_2$.

Membrane potential was measured using a FLIPR membrane potential assay kit (blue) from Molecular Devices (Sunnyvale, CA), as described previously.[88] The dye was reconstituted with assay buffer of composition (mM): NaCl 145, HEPES 22, $Na_2HPO_4$ 0.338, $NaHCO_3$ 4.17, $KH_2PO_4$ 0.441, $MgSO_4$ 0.407, $MgCl_2$ 0.493, $CaCl_2$ 1.26, glucose 5.56 (pH 7.4, osmolarity 315 ± 5). Prior to the assay, cells were loaded with 90 μL/well of the dye solution without removal of the L-15, giving an initial assay volume of 180 μL/well. Plates were then incubated at 37 °C at ambient $CO_2$ for 60 min. Fluorescence was measured using a FlexStation 3 (Molecular Devices) microplate reader with cells excited at a wavelength of 530 nm and emission measured at 565 nm. Baseline readings were taken every 2 s for 60−120 s, after which either drug or vehicle was added in a volume of 20 μL. The background fluorescence of cells without dye or dye without cells was negligible. Changes in fluorescence were expressed as a percentage of baseline fluorescence after subtraction of the changes produced by vehicle addition. Drug solutions were made up in assay buffer containing 0.01% BSA (Sigma) and 1% DMSO, thus the final concentration of dimethyl sulfoxide was always 0.1%.

Data were analyzed with PRISM (GraphPad Software Inc., San Diego, CA), using four-parameter nonlinear regression to fit concentration−response curves. In all plates, a maximally effective concentration of CP 55,940 (1 μM, Cayman Chemical, Ann Arbor, MI) was added to allow for normalization between assays.

**In Vivo Pharmacological Assessment of SCs.** Two cohorts of four adult male Long Evans rats (Animal Resources Centre, Perth, Australia) initially weighing between 168 and 186 g were used for biotelemetric assessment of body temperature and heart rate changes following each compound. The rats were singly housed in an air-conditioned testing room (22 ± 1 °C) on a 12 h reverse light/dark cycle (lights on from 21:00 to 09:00). Standard rodent chow and water were provided ad libitum. All experiments were approved by The University of Sydney Animal Ethics Committee.

Biotelemetry transmitters (TA11CTA-F40, Data Sciences International, St. Paul, MN) were implanted as previously described.[27] Briefly, following anesthetization (isoflurane, 3% induction, 2% maintenance) a rostro-caudal incision was made along the midline of the abdomen, and a biotelemetry transmitter (TA11CTA-F40, Data Sciences International, St. Paul, MN) was placed in the peritoneal cavity according to the manufacturers protocol. The wound was sutured closed and the rats were allowed 1 week of recovery before data collection.

The rats were habituated over multiple days to injections of vehicle (5% EtOH, 5% Tween 80, 90% physiological saline) at a set time of day (11:00 am). Each cohort then received injections of each compound at the same time of day in an ascending dose sequence (0.1, 0.3, 1, mg/kg). This ascending sequence reduces the risk posed to the animals in assessing hitherto untested compounds, and the use of multiple cohorts limits the potential development of tolerance to the compound. Two washout days were given between each dose. If only a modest or negligible hypothermic response was seen at 1 mg/kg, then a further 3 mg/kg dose of the compound was given. At least two washout days were given between each dose.

For the antagonist studies (Figure 6), the third and fourth cohort of drug-naïve rats were used for each compound, with a 48 h washout period between each dose. Each cohort received injections of either vehicle, $CB_1$ antagonist (rimonabant, 3 mg/kg), or $CB_2$ antagonist (SR144528, 3 mg/kg), followed by 5F-AMB (3 mg/kg) or MDMB-FUBINACA (1 mg/kg). The vehicle or antagonist injections were given to rats 30 min prior to the 5F-AMB or MDMB-FUBINACA injections.

Data for heart rate and body temperature was gathered continuously at 1000 Hz and organized into 15 min bins using Dataquest A.R.T. software (version 4.3, Data Sciences International, St. Paul, MN), and analyzed using PRISM (Graphpad Software Inc., San Diego, CA).

We calculated the area between baseline and drug-treatment body temperature curves for each rat as a measure of compound potency. Briefly, for any time point, the area between baseline data points ($B_t$) and drug-treatment data points ($D_t$) and the subsequent time points ($B_{t+1}$ and $D_{t+1}$) forms a trapezoid, the area of which can be calculated via the formula:

$$\text{area} = \frac{(B_t - D_t) + (B_{t+1} - D_{t+1})}{2}$$

These areas were summed from the time of injection to 6 h postinjection. MDMB-FUBINACA and 5F-AMB AUC data were compared at each dose level with independent samples $t$ tests.

For the antagonist studies, the area between the vehicle−vehicle baseline and the vehicle−SC (i.e., vehicle−5F-AMB or vehicle−MDMB-FUBINACA), rimonabant-SC, and SR144528-SC treatments was calculated over a 3 h time period postinjection of SC. These areas were analyzed using a one-way repeated measures ANOVA with planned Dunnet's contrasts comparing the antagonist areas to the vehicle-drug area.


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## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschemneuro.6b00137.

Table of compound names, CAS numbers, and relevant references; selected $^{1}H$ and $^{13}C$ NMR spectra (PDF)

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### Author Contributions

○S.D.B. and M.L. contributed equally.

### Author Contributions

The synthesis, purification, and chemical characterization of compounds **10**−**25** was carried out by S.D.B., M.L., and J.B.C.M., and overseen by M.K. M.C. and S.S. designed and conducted all in vitro pharmacological studies, and data analysis was performed by M.C., S.S., S.D.B., M.G., and J.S. M.S. made and characterized the $CB_1$ and $CB_2$ cells. All behavioral pharmacology was performed by R.K. with direction from I.S.M. The manuscript was drafted by S.D.B. with contributions from M.L., J.S. R.C.K., M.G., M.C., I.S.M., and M.K. All authors have given approval to the final version of the manuscript.


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### Notes

The authors declare no competing financial interest.

## ■ ABBREVIATIONS

CB, cannabinoid; FLIPR, fluorometric imaging plate reader; GIRK, G-protein-gated inwardly rectifying $K^+$ channels; ip, intraperitoneal; NMR, nuclear magnetic resonance; pi, post-injection; SAR, structure−activity relationship; SC, synthetic cannabinoid; $\Delta^9$-THC, $\Delta^9$-tetrahydrocannabinol; TLC, thin layer chromatography

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